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(71) Applicant *(for all designated States except US)*: THE MINISTER OF AGRICULTURE FISHERIES & FOOD [GB/GB]; Whitehall Place, London SW1A 2HH (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: JARRETT, Paul [GB/GB]; 14 Home Furlong, Wellesbourne, Warwickshire CV35 9TW (GB). ELLIS, Deborah, June [GB/GB]; 7 Cooke Close, Warwick, Warwickshire CV34 5YG (GB). MORGAN, James, Alun, Wynne [GB/GB]; Pen-Y-Goruf Farm, Gorof Road, Ystradgynlais, Swansea SA9 1TP (GB).

(74) Agent: SKELTON, S., R.; D/IPR, Formalities Section (Procurement Executive), Poplar 2, MOD Abbey Wood #19, P.O. Box 702, Bristol BS12 7DU (GB).

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(57) Abstract

A method for killing pests (e.g. insects) comprising administering material from *Xenorhabdus* species (e.g. *X. nematophilus*) such as cells or supernatants orally to the pests, either alone or in conjunction with *Bacillus thuringiensis* or pesticidal materials derived therefrom. Also disclosed is an isolated pesticidal agent (and compositions comprising the same) characterised in that it is obtainable from cultures of *X. nematophilus* or mutants thereof, has oral pesticidal activity against *Pieris brassicae*, *Pieris rapae* and *Plutella xylostella*, is substantially heat stable to 55 °C, is proteinaceous, acts synergistically with *B. thuringiensis* cells as an oral pesticide and is substantially resistant to proteolysis by trypsin and proteinase K. DNA encoding pesticidal activity is also disclosed.

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PESTICIDAL AGENTS

The present invention relates to materials, agents and compositions having pesticidal activity which derive from bacteria, and more particularly from *Xenorhabdus* species. The invention further relates to organisms and methods employing such compounds and compositions.

There is an ongoing requirement for materials, agents, compositions and organisms having pesticidal activity, for instance for use in crop protection or insect-mediated disease control. Novel materials are required to overcome the problem of resistence to existing pesticides. Ideally such materials are cheap to produce, stable, have a high toxicity (either when used alone or in combination) and are effective when taken orally by the pest target. Thus any invention which provided materials, agents, compositions or organisms in which any of these properties was enhanced would represent a step forward in the art.

Xenorhabdus spp. in nature are frequently symbiotically associated with a nematode host, and it is known that this association may be used to control pest activity. For instance, it is known that certain *Xenorhabdus spp*. alone are capable of killing an insect host when injected into the host's hemocoel.

In addition, one extracellular insecticidal toxin from *Photorhabdus luminescens* has been isolated (this species was recently removed from the genus *Xenorhabdus*, and is closely related to the species therein). This toxin is not effective when ingested, but is highly toxic when injected into certain insect larvae (see Parasites and Pathogens of Insects Vol.2, Eds. Beckage, N. E. et al., Academic Press 1993).

Also known are certain low-molecular weight heterocyclic compounds from *P.luminescens* and *X.nematophilus* which have antibiotic properties when applied intravenously or topically (see Rhodes, S.H. et al., PCT WO 84/01775).

Unfortunately none of these prior art materials have the ideal pesticide characteristics discussed above, and in particular, they do not have toxic activity when administered orally.

The present invention provides pesticidal agents and compositions from *Xenorhabdus* species, organisms which produce such compounds and compositions, and methods which employ these agents, compositions and organisms, that alleviate some of the problems with the prior art.

According to one aspect of the present invention there is disclosed a method of killing or controlling insect pests comprising administering cells from *Xenorhabdus* species or pesticidal materials derived or obtainable therefrom, orally to the pests.

A PCT application of CSIRO published as WO 95/00647 discloses an apparently toxic protein from *Xenorhabdus nematophilus*; however no details of the protein's toxicity are given, and certainly there is no disclosure of its use as an oral insecticide.

Thus the invention provides an insecticidal composition adapted for oral administration to an insect, which composition comprises a pesticidal material obtainable from a *Xenorhabdus* species, or a pesticidal fragment thereof, or a pesticidal variant or derivative of either of these.

The composition may in fact comprise cells of *Xenorhabdus* or alternatively supernatant taken from cultures of cells of *Xenorhabdus* species. However, the composition

preferably comprises toxins isolable from *Xenorhabdus* as illustrated hereinafter. Toxic activity has been associated with material encoded by the nucleotide sequence of Figure 2. Thus, the composition suitably comprises a pesticidal material which is encoded by all or part of the nucleotide sequence of Figure 2. Pesticidal fragments as well as variants or derivatives of such toxins may also be employed.

The sequence of Figure 2 is of the order of 40kb in length. It is believed that this sequence may encode more than one protein, each of which may regulate or be insecticidal either alone or when presented together. It is a matter of routine to determine which parts are necessary or sufficient for insecticidal activity.

As used herein the term ``variant'' refers to toxins which have modified amino acid sequence but which share similar activity. Certain amino acids may be replaced with different amino acids without altering the nature of the activity in a significant way. The replacement may be by way of ``conservative substitution'' where an amino acid is replaced with an amino acid of broadly similar properties, or there may be some non-conservative substitutions. In general however, the variants will be at least 60% homologous to the native toxin, suitably at least 70% homologous and more preferably at least 90% homologous.

The term ``derivative'' relates to toxins which have been modified for example by chemical or biological methods.

These toxins are novel, and they and the nucleic acids which encode them form a further aspect of the invention.

A preferred *Xenorhabdus* species is the bacteria *X.nematophilus*. Particular strains of *X.nematophilus* which are useful in the context of the invention are

ATTC 19061 strain, available from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland (NCIMB). In addition, suitable strains include two novel strains of *Xenorhabdus* which were deposited at the NCIMB on 10 July 1997 and were designated with repository numbers NCIMB 40886 and NCIMB 40887. These latter strains form a further aspect of the invention.

All strains have common characteristics as set out in the following Table 1.

Table 1

	Strains		
Characteristics	ATCC 19061	NCIMB 40887	NCIMB 40886
Gram strain	negative	negative	negative
Shape/size	rods up to 4µm long	rods up to 4µm long	rods up to 4µm long
Motile	Yes	Yes	Yes
Bioluminescent	No	No	No
Colour on NBTA*	blue	blue	blue
insecticidal on ingestion by insects	yes	yes	yes
Production of Antibiotics	yes	yes	yes
Resistant to ampicillin (50µg/ml)	yes	yes	yes
colony morphology/ colour	circular convex cream	circular convex cream	circular convex cream

*NBTA (Oxoid nutrient agar containing 0.0025% bromothymol blue and 0.004% tetrazolium chloride)

Preferably the pest target is an insect, and more preferably it is of the order Lepidoptera, particularly

Pieris brassicae, *Pieris rapae*, or *Plutella xylostella* or the order *Diptera*, particularly *Culex quinquefaciatus*.

In a preferred embodiment of the invention, cells from *Xenorhabdus* species or agents derived therefrom are used in conjunction with *Bacillus thuringiensis* as an oral pesticide.

In further embodiments, rather than using *Bacillus thuringiensis* itself, pesticidal materials obtainable from *B.thuringiensis* (e.g. delta endotoxins or other isolates) are used in conjunction with *Xenorhabdus* species.

The term 'obtainable from' is intended to embrace not only materials which have been isolated directly from the bacterium in question, but also those which have been subsequently cloned into and produced by other organisms.

Thus the unexpected discovery that bacteria of the genus *Xenorhabdus*(and materials derived therefrom) have pesticidal activity when ingested, and that such bacteria and materials can be used advantageously in conjunction with *B.thuringiensis* (and toxins or materials derived therefrom), forms the basis of a further aspect of the present invention. The pesticidal activity of *B.thuringiensis* isolates alone have been well documented. However, synergistic pesticidal activity between such isolates and bacteria of the *Xenorhabdus* species (or materials derived therefrom) has not previously been demonstrated.

In still further embodiments of the invention, culture supernatant taken from cultures of *Xenorhabdus* species, particularly *X. nematophilus*, is used in place of cells from *Xenorhabdus* species in the methods above.

All of these methods can be employed, inter alia, in pest control.

The invention also makes available pesticidal compositions comprising cells from *Xenorhabdus* species, preferably *X.nematophilus*, in combination with *B. thuringiensis*. As with the methods above, a pesticidal toxin from *B.thuringiensis* (preferably a delta endotoxin) may be used as an alternative to *B.thuringiensis* in the compositions of the present invention

Likewise, culture supernatant taken from cultures of *Xenorhabdus* species, preferably, *X.nematophilus* may be used in place of cells from *Xenorhabdus* species.

Such compositions can be employed, *inter alia*, for crop protection eg. by spraying crops, or for livestock protection. In addition, compositions of the invention may be used in vector control.

The invention further encompasses novel pesticidal agents which can be isolated from *Xenorhabdus spp*. Techniques for isolating such agents would be understood by the skilled person.

In particular, such techniques include the separation and identification of toxin proteins either at the protein level or at the DNA level.

The applicants have cloned and partially sequenced a region of DNA from *Xenorhabdus* NCIMB 40887 which region codes for insecticidal activity and this is shown as Figure 2 (SEQ ID NO. 1) hereinafter. Thus in a preferred embodiment the invention also provides a toxin which is encoded by DNA of SEQ ID No. 1 or a variant or fragment thereof.

The invention also provides a recombinant DNA which encodes such a toxin. The recombinant DNA of the invention may comprise the sequence of Figure 2 or a variant or fragment thereof. Other DNA sequences may encode similar proteins as a result of the degeneracy of the genetic code. All such sequences are encompassed by the invention.

The sequence provided herein is sufficient to allow probes to be produced which can be used to identify and subsequently to extract DNA of toxin genes. This DNA may then be cloned into vectors and host cells as is understood in the art.

DNA which comprises or hybridises with the sequence of Figure 2 under stringent conditions forms a further aspect of the invention.

The expression ``hybridises with'' means that the nucleotide sequence will anneal to all or part of the sequence of Figure 2 under stringent hybridisation conditions, for example those illustrated in ``Molecular Cloning'', A Laboratory Manual'' by Sambrook, Fritsch and Maniatis, Cold Spring Habor Laboratory Press, Cold Spring Harbor, N.Y.

The length of the sequence used in any particular analytical technique will depend upon the nature of the technique, the degree of complementarity of the sequence, the nature of the sequence and particularly the GC content of the probe or primer and the particular hybridisation conditions employed. Under high stringency, only sequences which are completely complementary will bind but under low stringency conditions, sequences which are 60% homologous to the target sequence, more suitably 80% homologous, will bind. Both high and low stringency conditions are encompassed by the term ``stringent conditions'' used herein.

Suitable fragments of the DNA of Figure 2, i.e. those which encode pesticidal agents may be identified using standard techniques. For example, transposon mutagenesis techniques may be used, for example as described by H.S. Siefert et al., Proc. Natl. Acad. Sci. USA, (1986) 83, 735-739. Vectors such as the cosmid cHRIMI, can be mutated using a variety of transposons and then screened for loss of insectidal activity. In this way regions of DNA encoding proteins responsible for toxic activity can be identified.

For example, the mini-transposon mTn3(HIS3) can be introduced into a toxic *Xenorhabdus* clone such as cHRIM1, hereinafter referred to as `clone 1', by electroporating cHRIM1 DNA into *E.coli* RDP146(pLB101) and mating this strain with *E.coli* RDP146(pOX38), followed by *E. coli* NS2114Sm. The final strain will contain cHRIM1DNA with a single insertion of the transposon mTn3(HIS3). These colonies can be cultured and tested for insecticidal activity as described in Example 8 hereinafter. Restriction mapping or DNA sequencing can be used to identify the insertion point of mTn3(HIS3) and hence the regions of DNA involved in toxicity. Similar approached can be used with other transposons such as Tn5 and mTn5.

Site directed mutagenesis of cHRIM1 as outlined in ``Molecular Cloning, A Laboratory Manual'' by Maniatis, Fritsch and Sambrook, (1982) Cold Spring Harbor, can also be used to test the importance of specific regions of DNA for toxic activity.

Alternatively, subcloning techniques can be used to identify regions of the cloned DNA which code for insecticidal activity. In this method, specific smaller fragments of the DNA are subcloned and the activity determined. To do this, cosmid DNA can be cut with a suitable restriction enzyme and ligated into a compatible

restriction site on a plasmid vector, such as pUC19. The ligation mix can be transformed into *E. coli* and transformed clones selected using a selection marker such as antibiotic resistance, which is coded for on the plasmid vector. Details of these techniques are described for example in Maniatis et al, supra, (see p390-391) and Methods in Molecular Biology, by L.G. Davies, M.D. Dibner and J.F. Battey, Elsevier, (see p222-224).

Individual colonies containing specific cloned fragments can be cultured and tested for activity as described in Example 8 hereinafter. Subclones with insecticidal activity can be further truncated using the same methodology to further identify regions of the DNA coding for activity.

The invention also discloses an isolated pesticidal agent characterised in that the agent is obtainable from cultures of *X. nematophilus* or variants thereof, has oral pesticidal activity against *Pieris brassicae, Pieris rapae* and *Plutella xylostella*, is substantially heat stable to 55°C, is proteinaceous, acts synergistically with *B.thuringiensis* cells as an oral pesticide and is substantially resistant to proteolysis by trypsin and proteinase K.

By 'substantially heat stable to 55°C' is meant that the agent retains some pesticidal activity when tested after heating the agent in suspension to 55°C for 10 minutes, and preferably retains at least 50% of the untreated activity.

By 'substantially resistant to proteolysis' is meant that the agent retains some pesticidal activity when exposed to proteases at 30°C for 2 hours and preferably retains at least 50% of the untreated activity.

By 'acts synergistically' is meant that the activity of the combination of components is greater than one might expect from the use of the components individually. For example, when used in conjunction with *B.thuringiensis* cells as an oral pesticide, the concentration of B. thuringiensis cellular material necessary to give 50% mortality in a P.brassicae when used alone is reduced by at least 80% when it is used in combination the agent at a concentration sufficient to give 25% mortality when the agent is used alone.

It has been found that the activity of the material is retained by 30 kDa cut-off filters but is only partly retained by 100 kDa filters.

Preferably the agent is still further characterised in that the pesticidal activity is lost through treatment at 25°C with sodium dodecyl sulphate (SDS - 0.1% 60 mins) and acetone (50%, 60 mins).

Clearly the characterising properties of the isolated agent described above can be utilised to purify it from, or enrich its concentration in, *Xenorhabdus* species cells and culture medium supernatants. Methods of purifying proteins from heterogenous mixtures are well known in the art (eg. ammonium sulphate precipitation, proteolysis, ultrafiltration with known molecular weight cut-off filters, ion-exchange chromatography, gel filtration, etc.). The oral pesticidal activity provides a convenient method of assaying the level of agent after each stage, or in each sample of eluent. Such methodology does not require inventive endeavour by those skilled in the art.

The invention further discloses oral pesticidal compositions comprising one or more agents as described above. Such compositions preferably further comprise other pesticidal materials from non-*Xenorhabdus* species.

These other materials may be chosen such as to have complementary properties to the agents described above, or act synergistically with it.

Preferably the oral pesticidal composition comprises one or more pesticidal agents as described above in combination with *B. thuringiensis* (or with a toxin derived therefrom, preferably endotoxin).

Recombinant DNA encoding said proteins also forms a further aspect of the invention. The DNA may be incorporated into an expression vector under the influence of suitable control elements such as promoters, enhancers, signal sequences etc. as is understood in the art. These expression vectors form a further aspect of the invention. They may be used to transform a host organism so as to ensure that the organism produces the toxin.

The invention further makes available a host organism comprising a nucleotide sequence coding for a pesticial agent as described above.

Methods of cloning the sequence for a characterised protein into a host organism are well known in the art. For instance the protein may be purified and sequenced: as activity is not required for sequencing, SDS gel electrophoresis followed by blotting of the gel may be used to purify the protein. The protein sequence can be used to generate a nucleotide probe which can itself be used to identify suitable genomic fragments from a *Xenorhabdus* gene library. These fragments can then be inserted via a suitable vector into a host organism which can express the protein. The use of such general methodology is routine and non-inventive to those skilled in the art. Such techniques may be applied to the production of *Xenorhabdus* toxins other than those encoded by the sequence of Figure 2.

It may be desirable to manipulate (eg. mutate) the agent by altering its gene sequence (and hence protein structure) such as to optimise its physical or toxicological properties.

It may also be desirable for the host to be engineered or selected such that it also expresses other proteinaceous pesticidal materials (eg. delta- endotoxin from *B. thuringiensis*). Equally it may be desirable to generate host organisms which express fusion proteins composed of the active portion of the agent plus these other toxicity enhancing materials.

A host may be selected for the purposes of generating large quantities of pesticidal materials for purification e.g. by using *B.thuringiensis* transformed with the agent-coding gene. Preferably however the host is a plant, which would thereby gain improved pest-resistance. Suitable plant vectors, eg. the Ti plasmid from *Agrobacterium tumefaciens*, are well known in the art. Alternatively the host may be selected such as to be directly pathogenic to pests, eg. an insect baculovirus.

The teaching and scope of the present invention embraces all of these host organisms plus the agents, mutated agents or agent-fusion materials which they express.

Thus the invention makes available methods, compositions, agents and organisms having industrially applicable pesticidal activity, being particularly suited to improved crop protection or insect-mediated disease control.

The methods, compositions and agents of the present invention will now be described, by way of illustration only, through reference to the following non-limiting examples and figures. Other embodiments falling within

the scope of the invention will occur to those skilled in the art in the light of these.

FIGURE

Figure 1 shows the variation with time of the growth of *X. nematophilus* ATCC 19061 and activity of cells and supernatants against *P. brassicae* as described in Example 3.

Figure 2 shows the sequence of a major part of a cloned toxin gene from *Xenorhabdus*.

Figure 3 shows a comparison of the restriction maps of cloned toxin genes from two strains of *Xenorhabdus* (clone 1 above and clone 3 below).

EXAMPLES

Example 1 - Use of *X. nematophilus* cells as an oral insecticide

CELL GROWTH: A subculture of *X.nematophilus* (ATCC 19061, Strain 9965 available from the National Collections of Industrial and Marine Bacteria, Aberdeen, Scotland) was used to inoculate 250 ml Erlenmeyer flasks each containing 50 ml of Luria Broth containing 10g tryptone, 5g yeast extract and 5g NaCl per litre. Cultures were grown in the flasks at 27°C for 40hrs on a rotary shaker.

PRODUCTION OF CELL SUSPENSION: Cultures were centrifuged at 5000 x g for 10 mins. The supernatants were discarded and the cell pellets washed once and resuspended in an equal volume of phosphate buffered saline (8g NaCl, 1.44g Na_2HPO_4 and 0.24g of KH_2PO_4 per litre) at pH 7.4.

ACTIVITY OF CELL SUSPENSION TO INSECTS: The bioassays were as follows: *P. brassicae*: The larvae were allowed to feed on an artificial agar-based diet (as described by David and Gardiner (1965) London Nature, 207, 882-883) into which a series of dilutions of cell suspension had been incorporated. The bioassays were performed using a series of 5 doses with a minimum of 25 larvae per dose. Untreated and heat-treated (55°C for 10 minutes) cells were tested. Mortality was recorded after 2 and 4 days with the temperature maintained at 25°C.

	LC50 cells/g diet	
Treatment	2 days	4 days
Untreated	5.9×10^5	9.8×10^4
Treated 55°C	7.1×10^5	1.4×10^5

Aedes aegypti: The larva were exposed to a series of 5 different dilutions of cell suspension in deionised water. The biosassays were performed using 2 doses per dilution of 50 ml cell suspension in 9.5cm plastic cups with 25 second instar larvae per dose. Untreated and heat-treated (55°C or 80°C for 10 minutes) cells were tested. Mortality was recorded after 2 days with the temperature maintained at 25°C.

	LC50 cells/ml
Treatment	2 days
Untreated	5.1×10^6
Treated 55°C	7.4×10^6
Treated 80°C	$> 10^8$

Culex quinquefaciatus: The larvae were exposed to a single concentration cell suspension containing 4×10^7 cells/ml. The biosassays were performed using 2 50 ml cell suspensions in 9.5 cm plastic cups with 25 second instar larvae per cup. Untreated and heat-treated (55°C or 80°C for 10 minutes) cells were tested. Mortality was

recorded after 2 days with the temperature maintained at 25°C.

Treatment	% Mortality 2 days
Untreated	100
Treated 55°C	100
Treated 80°C	0

Thus these results clearly show that cells from *X. nematophilus* are effective as an oral insecticide against a number of insect species (and are particularly potent against *P.brassicae*). The insecticidal activity is not dependent on cell viability (i.e is largely unaffected by heating to 55°C which reduces cell viability by >99.99%) but is much reduced by heating to 80°C, which denatures most proteins.

Example 2 - Use of *X.nematophilus* supernatant as an oral insecticide

CELL GROWTH: Cultures were grown as in Example 1.

PRODUCTION OF SUPERNATANT: Cultures were centrifuged twice at 10000g for 10 mins. The cell pellets were discarded.

ACTIVITY OF SUPERNATANT TO INSECTS: The Bioassay was as follows:
Activity against neonate *P. brassicae* and two day old *Pieris rapae* and *Plutella xylostella* larvae was measured as for *P. brassicae* in Example 1, but using a series of untreated dilutions of supernatant in place of of cell supensions and with mortality being recorded after 4 days only.

Insect species	LC50 (μl supernatant/g diet) 4 days
P. brassicae	22
P. rapae	79
P. xylostella	135

In addition, size-reducing activity (62% reduction in 7 days) against *Mamestra brassicae* was detected in larvae fed on an artificial diet containing *X. nematophilus* supernatant (results not shown).

Thus these results clearly show that the supernatant from *X. nematophilus* culture medium is effective as an oral insecticide against a number of insect species, and are particularly potent against *P. brassicae*.

The heating of supernatants to 55°C for 10 minutes caused a partial loss of activity while 80°C caused complete loss of activity. Activity was also completely lost by treatment with SDS (0.1%w/v for 60 mins) and Acetone (50% v/v for 60 mins) but was unaffected by Triton X-100 (0.1% 60 mins), non-diet P40 (0.1% 60 mins), NaCl (1 M for 60 mins) or cold storage at 4°C or -20°C for 2 weeks. All of these properties are consistent with a proteinaceous agent.

The general mode of action of *X. nematophilus* cells and supernatants i.e. reduction in larval size and death within 2 days at high dosages, and other properties, eg. temperature resistence, appear to be similar suggesting a single agent or type of agent may be responsible for the oral insecticide activity activities of both cells and supernatants.

Example 3 - Timescale for appearance of ingestable insecticidal activity

CELL GROWTH: 1ml of an overnight culture of *X. nematophilus* was used to inoculate an Erlenmeyer flask. Cells were then cultured as in Example 1. Growth was estimated by measuring the optical density at 600 nm.

PRODUCTION OF CELL SUSPENSION AND SUPERNATANTS: These were produced as in Examples 1 and 2.

ACTIVITY OF CELLS AND SUPERNATANTS AGAINST *P. BRASSICAE*: The cell suspension bioassay was carried out as in Example 1, but using a single dose of suspended cells equivalent to 50 μl of broth/g diet and measuring mortality after 2 days. The cell supernatant bioassay was carried out as in Example 2, but using a single dose equivalent to 50 μl supernatant/g diet (i.e. more than twice the LC50) and measuring mortality after 2 days.

The results are shown in Fig. 1. Thus these results clearly show that cells taken from *X. nematophilus* culture medium are highly effective as an oral insecticide against *P. brassicae* after only 5 hours, and supernatants are highly effective after 20 hours. Although some slight cell lysis was observed in the early stages of growth, no significant cell lysis was observed after this point demonstrating that the supernatant activity may be due to an authentic extracellular agent (as opposed to one released only after cell breakdown).

Example 4 - Synergy between *X. nematophilus* cells and *B.thuringiensis* powder preparations

CELL GROWTH AND SUSPENSION: *X. nematophilus* cells were grown and suspended as in Example 1. *B. thuringiensis* strain HD1 (from *Bacillus* Genetic Stock Centre, The Ohio State University, Columbus, Ohio 43210, USA) was cultured, harvested and formulated into a powder as described by Dulmage *et al.*(1970) J. Invertebrate Pathology 15, 15-20.

ACTIVITY OF *X. NEMATOPHILUS* CELLS AND *B. THURINGIENSIS* POWDER AGAINST *P. BRASSICAE*: The bioassays was carried out using *X. nematophilus* and *B. thuringiensis* in combination or using *B. thuringiensis* cell powder alone. Bioassays were carried out as in Example 1 but with various dilutions of *B. thuringiensis* powder in place of *X. nematophilus*. For the combination experiment, a constant dose of *X. nematophilus* cell suspension sufficient to give 25% mortaility was also added to the diet. Mortality was recorded after 2 days.

Bioassay	LC50 (μg Bt powder/g diet) 2 days
B.t. alone	1.7
B.t. plus *X.nematophilus*	0.09

These results clearly demonstrate the synergism between *X. nematophilus* cells and *B. thuringiensis* powder when acting as an oral insecticide against *P. brassicae*.

Example 5 - Synergy between of *X.nematophilus* supernatants and *B. thuringiensis* powder

CELL GROWTH AND PRODUCTION OF SUPERNATANTS: *X. nematophilus* cells were grown and supernatants prepared as in Example 2. *B. thuringiensis* was grown and treated as in Example 4.

ACTIVITY OF *X. NEMATOPHILUS* SUPERNATANTS AND Bt CELL POWDER AGAINST *P. BRASSICAE*:
The bioassays were carried out using *X. nematophilus* supernatants and *B. thuringiensis* in combination or using *B. thuringiensis* powder alone. The Bioassay against neonate *P. brassicae* and two day old *Pieris rapae* and *Plutella xylostella* larvae were measured as in Example 2 but with various dilutions of *B. thuringiensis* in place of *X. nematophilus*. For the combination experiment, a

constant dose of *X. nematophilus* supernatant sufficient to give 25% mortality was also added to the diet. Mortality was recorded after 4 days.

	LC_{50} (μg Bt powder/g) diet	
Insect species	Bt alone	Bt plus Xn
P. brassicae	1.4	0.12
P. rapae	2.5	0.26
P. xylostella	7.2	0.63

These results clearly demonstrate the synergism between *X.nematophilus* supernatants and *B.thuringiensis* powder when acting as an oral insecticide against several insect species. The fact that both *X. nematophilus* cells and supernatants demonstrate this synergism strongly suggests that a single agent or type of agent is responsible for the demonstrated activities.

Example 5 - Characterisation of insecticidal agent from *X.nematophilus* supernatant by proteolysis

CELL GROWTH AND PRODUCTION OF SUPERNATANTS: *X. nematophilus* cells were grown and supernatants prepared as in Example 2.

PROTEOLYSIS OF SUPERNATANT: Culture supernatant (50ml) was dialysed against 0.5 M NaCl (3 x 1 l) for 48 hours at 4°C. The volume of the supernatant in the dialysis tube was reduced five-fold by covering with polyethylene glycol 8000 (Sigma chemicals). Samples were removed and treated with either trypsin (Sigma T8253 = 10,000 units/mg) or proteinase K (Sigma P0390 = 10 units/mg) at a concentration of 0.1 mg protease/ml sample for 2 hours at 30°C.

ACTIVITY OF PROTEASE TREATED SUPERNATANT AGAINST *P. BRASSICAE*: The boassay against neonate *P. brassicae*

larvae was carried out by spreading 25 μl of each 'treatment' on the artificial agar-based diet referred to in Example 1 in a 4.5 cm diameter plastic pot. Four pots each containing 10 larvae were used for each treatment. Mortalities were recorded after 1 and 2 days. Controls using water only, trypsin (0.1 mg/ml) and proteinase K (0.1 mg/ml) were also tested in the same way.

	% Mortality	
Treatment	1 day	2 days
Untreated supernatant	60	100
Proteinase K treated supernatant	45	100
Trypsin treated supernatant	40	100
All controls (no supernatant)	0	0

Example 6

Entomocidal activity of other *Xenorhabdus*

Using the methodology of Examples 1 and 2, four different *xenorhabdus* strains were tested against insect pests. The results obtained were as follows:

I) Activity to *Pieris brassicae*

Strain deposit no/code	Cells 10^6/grm diet % mortality	Supernatant LC50 μl/gram of diet
NCIMB 40887	100	0.09
0014	100	0.52
0015	80	3.73
NCIMB 40886	100	0.05

It was found that entomocidal activity of cells and supernatant was reduced by more than 99% when all four strains were heated at 80°C for 10 minutes.

II) Activity to mosquitoes (*Aedes aegypti*)
Bacteria added at the rate of 10^7cells/ml of water

Strain deposit no/code	Cells 10^6/grm diet % mortality
NCIMB 40887	0
0014	40
0015	45
NCIMB 40886	95

Furthermore, all strains significantly reduced the growth of *Heliothis virescens*.

Example 7

Cloning of toxin genes from strains of *Xenorhabdus*

Total cellular DNA was isolated from NCIMB 40887 and ATCC 19061 using a Quiagen genomic purification DNA kit. Cells were grown in L borth (10g tryptone, 5g yeast extract and 5g NaCl per l) at 28°C with shaking (150rpm) to an optical density of 1.5 A_{600}. Cultures were harvested by centrifugation at 4000xg and resuspended in 3.5mls of buffer B1 (50mM Tris/HCl, 0.05% Tween 20, 0.5% Triton X-100, pH7.0) and incubated for 30 mins at 50°C. DNA was isolated from bacterial lysates using Quiagen 100/G tips as per manufacturers instructions. The resulting purified DNA was stored at -20°C in TE buffer (10mM Tris, 1mM EDTA, pH 8.0).

A representative DNA library was produced using total DNA of NCIMB 40887 and ATTC 19061 partially digested with the restriction enzyme *Sau3a*. Approximately 20μg of DNA from each strain was incubated at 37°C with 0.25 units of the enzyme. At time intervals of 10, 20, 30, 45 and 60 minutes, samples were withdrawn and heated at 65°C for 15 minutes. To visualise the size of the DNA fragments, the samples were electrophoresed on 0.5% w/v agarose gels.

The DNA samples which contained the highest proportion of 30 to 50kb fragments were combined and treated with 4 units of shrimp alkaline phosphatase (Boehringer) for 15 minutes at 37°C, followed by heat treatment at 65°C to inactivate the phosphatase.

The size selected DNA fragments were ligated into the BamH1 site of the cosmid vector SuperCos! (Stratagent) and packaged into the *Escherichia coli* strain XL Blue 1, using a Gigapack II packaging kit (Stratgene) in accordance with the manufacturers instructions.

To select for cosmid clones with entomocidal activity, individual colonies selected on L agar plates containing 25µg/ml ampicillin, were grown in L broth (containing 25µg/ml ampicillin) overnight at 28°C. Broth cultures (50µl) were individually spread onto the surface of insect diet contained in 4.5cm diameter pots, as described in Example 5. To each container 10 neonate *P. brassicae* larvae were added. Larvae were examined after 24, 72 and 96 hours recording mortality and size of surviving larvae. A total of 220 clones of NCIMB 40887 were tested, of which two were found to cause reduction in larval growth and death within 72 hours. Of 370 clones from ATTC 19061, one was found to cause larval death within 72 hours.

Example 8

Activity of cloned toxin genes to *Pieris brassicae*

The three active clones from Example 7 were grown in L broth, containing 25µg/ml ampicillin, for 24 hours at 28°C, on a rotary shaker at 150rpm. The activity of the toxin clones to neonate larvae were performed by incorporation of whole broth cultures into insect diet, as described in Example 1.

Clone No	Strain	LC50 (µl broth/g insect diet)
1	NCIMB 40887	13.03
2	NCIMB 40887	16.7
3	ATTC 19061	108.7
Control*		No effect at 100µl/g

*XL1 Blue *E. coli* broth

When *E. coli* toxin clones were heated at 80°C for 10 minutes and added to the diet at a rate of 100µl/g, no activity to larvae was detected. Highlighting the heat sensitivity of the toxins.

Example 9

Sequencing of the cloned toxin from NCIMB 40887

Cosmid DNA of the entomocidal clone 1 above from NCIMB 40887 was purified using the Wizard Plus SV DNA system (Promega) in accordance with the manufacturers instructions. A partial map of the cloned fragment was obtained using a range of restriction enzymes *Eco*R1, *Bam*H1, *Hind*III, *Sal*1 and *Sac*1 as shown in Figure 3. DNA sequencing was intiatiated from pUC18 and pUC19 based sub-clones of the cosmid, using the enzymes *Eco*R1, *Bam*H1, *Hind*III, *Eco*RV and *Pvu*II. Sequence gaps were filled using a primer walking approach on purified cosmid DNA. Sequence reactions were performed using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmmpliTaq DNA polymerase FS according to the manufacturers instructions. The samples were analysed on an ABI automated sequencer according to the manufacturers instructions. The major part of the DNA sequence for the cloned toxin fragment is shown in Figure 2.

Example 10

Restriction map of cloned toxin from clone 3

Cosmid DNA of the entomocidal clone 3 above was purified as described in Example 9. A restriction map of the cloned fragment was obtained using the restriction enzymes *Bam*H1, *Hind*III, *Sal*1 and *Sac*1 and this is shown in Figure 3. When compared with the map from clone 1 (Figure 3) it is clear that over the regions which overlap, the restriction maps are very similar. The only detectable difference between the two clones was a reduction in size of two *Hind*III fragments in clone 3, corresponding to the 11.4kb and 7.2kb *Hind*III fragments in clone 1 by approximately 2Kb and 200bp respectively. These results indicate the overall relatedness of the DNA region coding for toxicity in the two bacterial strains.

Example 11

Southern Blot Hybridisation Experiments

A 10.3kb *Bam*H1-*Sal*1 fragment of the DNA from clone 1 was used as a probe to hybidise to total *Hind*III digested DNA of the *Xenorhabdus* strains ATCC 19061, NCIMB 40886 and NCIMB 40887. Hybridisation was performed with 20ng/ml of DIG labelled DNA probe at 65°C for 18 hours. Filters were washed prior to immunological detection twice for 5 minutes with 2 x SSC (0.3M NaCl, 30mM sodium citrate, pH 7.0)/0.1% (w/v) sodium dodecyl sulphate at room temperature, and twice for 15 minutes with 0.1 x SSC (15mM NaClm 1.5 mM sodium citrate, pH 7.0) plus 0.1% sodium dodecyl sulphate at 65°C. The probe was labelled and experiments performed in accordance with manufacturers instructions, using a non-radioactive DIG DNA labelling and detection kit (Boehringer). The probe hybridised to a *Hind*III fragment of approximately 8kb in all three strains as well as an 11.4kb fragment in NCIMB 40887 and an approximate 9kb fragment in both NCIMB 40886 and ATCC 19061. These results show that strains NCIMB

40886 and ATCC 19061 contain DNA with close homology to the toxin gene of clone 1 above, confirming the similarity between the toxins produced by the three strains.

CLAIMS

1. An insecticidal composition adapted for oral administration to an insect comprising a pesticidal material obtainable from a *Xenorhabdus* species, or a pesticidal fragment thereof, or a pesticidal variant or derivative of either of these.

2. A composition according to claim 1 wherein the said pesticidal material comprises material encoded by the nucleotide sequence of Figure 2 or variant or fragment thereof, or a sequence which hybridises with said sequence.

3. A composition according to claim 1 or claim 2 which comprises cells of *Xenorhabdus*.

4. A composition as claimed in any one of the preceding claims which comprises supernatant taken from cultures of cells of *Xenorhabdus* species.

5. A composition according to any one of the preceding claims wherein the *Xenorhabdus* species is *Xenorhabdus nematophilus*.

6. A composition according to any one of claims 1 to 4 wherein the *Xenorhabdus* species is ATCC 19061, NCIMB 40886 or NCIMB 40887.

7. A composition as claimed in any one of the preceding claims which comprises a further pesticidal material not obtainable from *Xenorhabdus*.

8. A composition according to claim 7 wherein the said further pesticidal material comprises a material obtainable from *B. thuringiensis*.

9. A composition according to claim 8 which further comprises cells of *B. thuringiensis*.

10. A composition according to claim 8 wherein the pesticidal materials obtainable from *B.thuringiensis* comprises the delta endotoxin.

11. A composition according to any one of the preceding claims which further comprises an agriculturally acceptable carrier.

12. A composition according to claim 10 wherein the carrier comprises items of insect diet.

13. A method for killing or controlling insect pests, which method comprises administering to a pest or the environment thereof a composition according to any one of the preceding claims.

14. A method as claimed in claim 12 wherein the pests are insects from the order Lepidoptera or Diptera.

15. A microorganism comprising *Xenorhabdus* strain NCIMB 40886.

16. A microorganism comprising *Xenorhabdus* strain NCIMB 40887.

17. A pesticidal agent which comprises a a toxin comprising a protein which is encoded by DNA which includes SEQ ID No. 1 or a variant or fragment thereof.

18. An isolated pesticidal agent characterised in that it is obtainable from cultures of *X. nematophilus* or mutants thereof, has oral pesticidal activity against *Pieris brassicae*, *Pieris rapae* and *Plutella xylostella*, is substantially heat stable to 55°C, is proteinaceous, acts synergistically with *B. thuringiensis* cells as an

oral pesticide, and is substantially resistant to proteolysis by trypsin and proteinase K.

19. An isolated pesticidal agent as claimed in claim 18 further characterised in that the pesticidal activity is substantially destroyed by treatment with sodium dodecyl sulphate or acetone or heating to 80°C.

20. An isolated pesticidal agent as claimed in claim 18 or claim 19 further characterised in that the agent is an extracellular protein.

21. A recombinant DNA which encodes a pesticidal agent according to any one of claims 17 to 20.

22. A recombinant DNA of claim 21 which comprises the sequence of Figure 2 or a variant or fragment thereof.

23. A recombinant DNA which comprises or hybridises under stringent conditions with all or part of the sequence of Figure 2, and which encodes a pesticidal material.

24. An expression vector comprising a recombinant DNA according to any one of claims 21 to 23.

25. A host organism which has been transformed with an expression vector according to claim 24.

26. A host organism as claimed in claim 25 which has been engineered or selected such that it also expresses other pesticidal proteinaceous toxicity enhancing materials

27. A host organism comprising a nucleotide sequence coding for a fusion protein comprising a pesticidally active portion of an agent as claimed in any one of claims 17 to 20 in combination with other pesticidal proteinaceous toxicity enhancing materials.

28. A host organism as claimed in claim 27 wherein the pesticidal toxicity enhancing materials comprise delta-endotoxin from B. thuringiensis.

29. A host organism as claimed in any one of claims 25 to 289 wherein the host is a plant.

30. A host organism as claimed in any one of claims 25 to 28 wherein the host is a virus pathogenic to insects.

31. A fusion protein as expressed by a host as claimed in claim 27.

32. An pesticidal composition comprising one or more agents as claimed in any one of claims 17 to 20.

Fig.1.

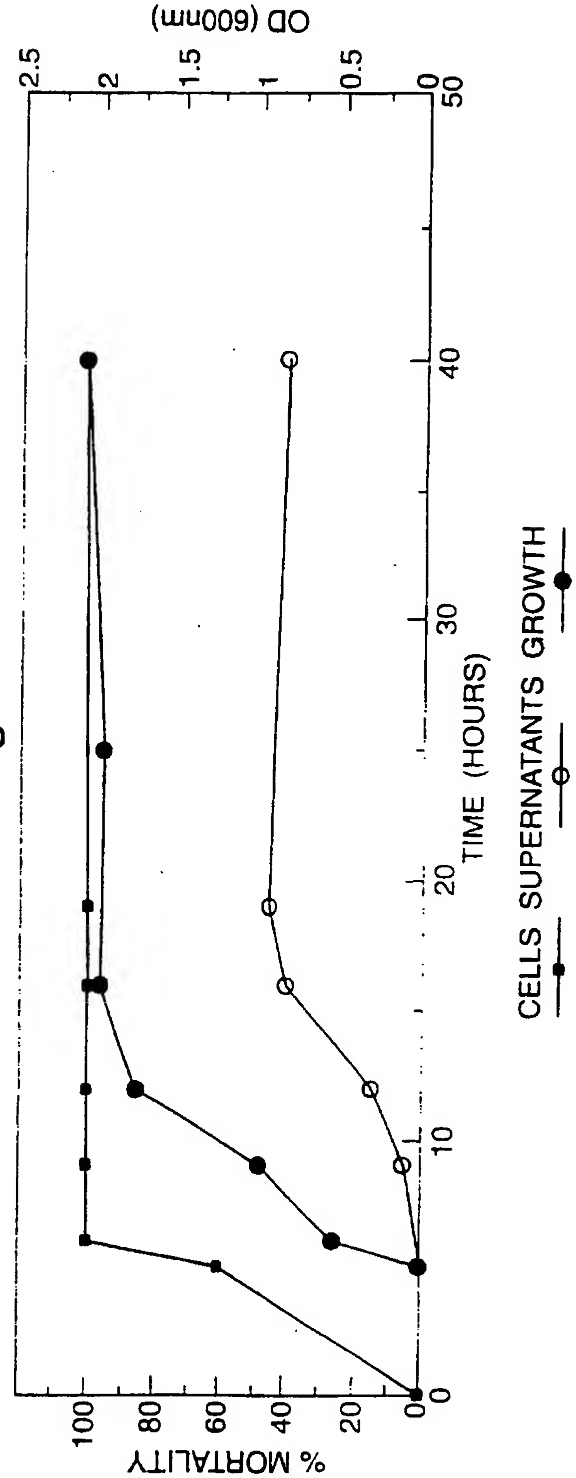
OD (600nm)
2.5
2
1.5
1
0.5
0
% MORTALITY
100
80
60
40
20
0
0
10
20
30
40
50
TIME (HOURS)
CELLS
SUPERNATANTS
GROWTH

Fig.2.

```
   1 TCCACAATTG CCGGAGAAAA TCAGTCGGGA ACTGCCGGTG ATTATTCGTC ACTTATTAAA
  61 CGAATTTGCC GACCAGAATA AGGCTAAAAA ACTGCTACAG GCGCAACGCG ACTCGAACGA
 121 AGCGTTAACG GTAAAGAGTC ATTCGGATCC GCTGTATCGC TTTTGTGGTT ATCTGGTGTC
 181 TGTCAATGAT ATGACCGGAA TGAAGATGGG CAATAAAAAC ATTAGCCCAC GAGCACCGAG
 241 ATTGTACTTG TATCATGCCT ATCTCTCTTT TATGGAAGCG CACGGCTTTG AACGTCCGTT
 301 AACACTGACT AAGTTTGGTG AATCCATCCC CAAGATTATG CTGGAATACC GGAAGGAGTA
 361 TCGAAAAGTG CGAACCAAGA AAGGCTATTC CTATAACGTG GAATTATCGG AAGAGGCCGA
 421 AGAATGGCTA CCGTCAGTGC CTGAGTGTCG AGACTTTAAA TCACCTGTAT AAAACTTTGA
 481 GCTTTAAGTC TGCACTCCAT ACACAACTTA AAATATCTAA TTGTATTTAA AAGAAAATAA
 541 TAGATGTATA GTTATTTTTT AACTATACAT AAGCTCTACA TGCTCTTCAT TCGTGTAAAA
 601 AATGGGTGAA CAGGTGATAC AGTCAGTGAA TATCATATTA ATTACCGTAA ACCCAGATGT
 661 AGCAAGGCTT TCAGGGAATT GTGCAGAGGG TGCATAACTG AGAGGGTGAA AAAGATTTTC
 721 AGGGGGGCTT ATGGCAGGTA AACAAAATCA GAAGCAAATA CCGTGCACAA TCTGGTTTTT
 781 ATTTTTTGGT ACTACCTCAA ATTAAAATGA TGTAATCATC TGATTTTATT TAAGAATAGA
 841 AGTTAATCAC AATTTCATTG ATGGACTTTC ATTCACACTG GTATAGATAA ATAATTCTGT
 901 TATATCCTGT TTCATTACGC ATTCATCAGG AGTGCTGTTA CAGGAGACAA GAATGTCACA
 961 CATCATTTAC TTGTCGTTAA AGGGCAAGAA GCAGGGTTTA ATTTCAGCGG GTTGTTCAAC
1021 GCCTGAATCA ATTGGAAATC GCTATCAAAA AGGACGTGAA GATCAAATAC AGGTATTGAG
1081 CCTGAATCAT TCGATGAGCC GTGACCAGAA TGTTAATCAT CAACCCGTCA GTTTTGTGAA
1141 ACCCATTGAT AAATCCTCTC CCCTGTTTGC TGGATGCCAG TTTTGTGCAT TACAGGACAA
1201 GCCAGATGGG ACAACTGGAG TTCTTTTATG AAATCAAGCT GACCAGTGCC ACGATTGTGG
1261 ATATTTCCTA TAATTATCCG GCATTCAATC AATGATAATG GTGCGATACC CCATGAAGTG
1321 GTGATGCTCG ATTATAAGTC CATTTCATGC AACCACATCG CCGCAGGACT TCGGGCTACA
1381 GCATACGCAA TTAGCCGGAA GTGAAGAAGC AAGCCGCTTT TATCTGGGGT CTCGAATGTT
1441 AAGCCACTTA AGAAGCCGCT GGTTGAAGAA ACCCCGGTAA AACCCGCTAA ACATCATGCC
1501 CGTTATCGTT GTGTGGATGA TGACGGCAAT CTTTTAACCG AACGCAAGTA TCGGGTTTGC
1561 CTGCCGGATG GTCAGATAAA AGAAGGAAAG ACTGATAAAC AAGGTTACAC CCAATGGCAT
1621 CTTACGGATG ACAAAAATAA ACTTGAATTT CATATTTTAA AGGATTAATA CCATGCCAGC
1681 CTATACCGTT CAGACAAAAA TAGAATCCAA CGTACCTGTT GAAAACCTGC TTTACGACTT
1741 AACCATTTAT CGTAAGGATG CAAAAGGAAA TTTCCATATC TTGCTTGATG TTTTTCAGGA
1801 GAAACTACAG AGTAATTATG AAACACAACA GCATATCACG CAGGAAATAG ACGACGATCT
1861 TTCTGTGATT TATATTATGC AAATTATGCT TCACCGCAAA CATGGCTCAA ATATATTTCC
1921 GGCACTGCAA ACCCATTTTA AGAAAATGTA TACCCTCGGT GAATTAACTT CCGGTAAAGC
1981 CTGTTCGGAG AAAAAACGGG AAAATGCCTG TTATTTTGAA AGTACAGTTG AAACAAAACC
2041 TGTCAGCGAC GGGGATAATA CCGTTGACTT AAATATCACT ATTCCTGAAC GACCTTTTAT
2101 TGCCAAAGAA TATCCCATTG GTCACCCACA CGATCCATTT GAAAAAAGTA AAATTGAATC
2161 ATAAATACAG GACAGGTTAT CGAAAAGAAT TTATCCGGAT CAAAATGGAG CAAGTTTATG
2221 TCAGGGCGCG AGCACACTAT TTTAGCTGCG TTTTTAAGAT GATTATCTCT TAATGTTCAG
2281 TTTTAATAGT GTTTTTATCG AGTGAAATTT AATCGCACAG GCAATTCTTT AGACTTTTAT
2341 AGAAAACTAA AGAATTAAAG AACAAGATTG ACATTTTAAG TTCAAATATT AATCAAAGTA
2401 TGCTCGCGCC CTGAGTTTAT GTGGCCCTGC CGCTTTTTTT TATTGCCTGC CAATAGATAG
2461 ACCAGATATT TATGAGCAAG CGGCACGAGA ATTATGGCAA TATGGCCGAA CTAAAATTGG
2521 TCAACTGGAA ATTAAGCCGG GTGAGGGTTG CCGACATCCT AAAGGTACTT TTTATAATCA
2581 ATATGGTGAA AGAATATCTG GGTTAGATTG GCTGACATTG GCAAGCCTAA GAGATTCAGA
2641 AAATATGATG ATGAGGTTGA TGATGAAGTA GCTGGTATTA CAATGTGGGG AAAATTGACA
2701 GAATGGTTTG AAAAATCAGG GTATGAAAAA GTATTTAGTA ATGTCGGCTT ATCCCATTCT
2761 AATATAAATG ACATAGTAAC TCTTAGTGAT TACTATAACA AAGGATATCA TGTTGTTACT
2821 TTGATTTCAG CAGGAATGTT ATCAGATTTT GGTGACATAG AAACATCAGG AAAAAATCAT
2881 TGGATAGTTT GGGAAGGAGT AGTAGAAAAC TATGAGAAAG AAAATATCAC AAATAATTCA
2941 GATCTGAATC AATATGTAAA TTTAAATCTG TTTTCATGGG GTAAAGTGGA ACATCAAATT
3001 AAAAAAAACA AATCACTAGA TTATGTACTC AACCATATTT TTTGAGGGTT GGTTTTTAAA
3061 CCAATGAAAT AACATGAAAA AAATATTAAT TATTTTTATT TTTTTACTTT ATGGTTGTGG
3121 TAATCCAACG CCAAAAGTTT TACCAAAATC AGAGTTTCTT CCTGATGCAG TGATAAATGA
3181 ACCATATCAG GCATCAATTA CCATCACAGG AGGTGCATTG AATGAAAAAA GCGTTTGGGT
3241 AAAAATTCAT CCTACTGGCT CAGGACTAAC ATGGAATCCA AAAGATAGTT CTTTCCTATA
3301 GGGTGGAAAA AAAGAAATAA GAAAAGATTA TCATCATATA AATATAACAG GTACCCCAAA
3361 GAAGACAGAA TTGATAAAAA TTGAAGTGGT AGGATTTACA TTGGGTACAA TGTACGCACG
3421 GAAAGAGTTC ACTATAAATT ATACTATAAA AGTAAGGGAA TAATTGTCAC TATCAGAATG
3481 GTGATTTAAT TCGCCATTTT TATACTTTTG TATACTCTCT CAACATAATC AGGATTCTTT
```

Fig.2.

3541 CTTATTATTT TTCATGGTGC TAAAAACGTT TATTGCAAAA ATAAATTAAG TTAATCAGAT
3601 AAATTATCTG CATTACTGTT ATAATCGATA ACACGATAAC CTGACTTTCT GCCTGTTCTT
3661 ATGAACTCGA AGATAATCCT TTCTGAGCCT GAACGAATCA CATTGCAACC ACTCGCTTTG
3721 AATCACCCAC ACCGGGACAT TCGTACGCGA GGAACGGGTT TACTCATGCT TGCCAGAGGG
3781 AGCAAGCCGT CCCAGATCAC CGCTGAAATC GGATGCAGTC TCCGGGTTAT CTGTAATTGG
3841 GTTCACATGT GGCACAGATA GCGGGATTAT TCGGCGGTCA TGCCGGAGGC CGGTATCTCG
3901 CCATGACGCC TGACATGATT GCCACTGCGC TCGAAGCCGC CAGCGCAGAG TCCCTGACGT
3961 GCGTCGAAGC CAGGCAGGGT TTCCCTGCCT TGTACGCTTG AAACGCTGGC GAATACCCTG
4021 AAAAAACAGG GGCTCCCCTA TAAACGCCCC CGCCTGTCGC TTAAAAAAAG CGCAATAAAA
4081 CGGAGTTTGC TGAAAAATCC GCCTTGCTGA ATAAAATTAA GGCCGGAGCA CAGTCAGGAC
4141 ATTACCGTCT GGTCTATTTT GAGTTCTGGG GGCGTTAAAT TACACGGATA ACACGCTGTT
4201 TTACCAGACA ACGTCAGGCA GTATCACGCG AGATGACGTG ATTGATTTTT TAGAGCCGGT
4261 GGCCAGACAA GGGACAACCG CCTGACATTT TTAGTGTTGG ATAATGCGCG TATCCATCAC
4321 GGGATAGAGG AAAAAATCAG AAATGGCGGG TGACGAGAAC ACAACCTGTT TTTATTCTAT
4381 CTTCCCGCTT ACAGCCCAGA GCTGTATCTG ATTGAAATCG TCTGGAAACA GGCCAAATAC
4441 GACTGGCGAC GTTTTATCAC CTGGACTCAG GATACAATGG AATATGAGGT AAATACTTTA
4501 TTGAAAGGTT ATGGCGACCA ATTTGCAATT AACTTTTCTT GAGTACTTAG TAAGAATAGA
4561 GTCAGTCGAG GTTTTTTCAT TTCGGGTCGT GGGGATGATA CTGAAAATTT GTTTGTAATC
4621 TCTGAAAATT GCTGTTTCTG TGGCTACGTC TGTCTTTTGG GATATTGTTT CCATCAAGTC
4681 TGTCAACATA CTGTTAAGTT AGATGTTGAT AAAAGAGACT GAATTATAAT ACAAAACAAT
4741 AAATCACTTG GACAATATTT TATTTCACAT GAGACATTAA GGTTGATTTT CCCAATCTGG
4801 TCAGTTATAA CCGAATAAGG ATCTTGAAAA ATCATGGGAT CTTACTTTTA TCAAATGAAG
4861 TTAACGTAAA AGTTGATAAA GAAAATTATT TAATTCTAAG TGCCGTTGGC ATAAATATTT
4921 TGTGTTTTGT TAATGAATGA ATAACCAGGT AAGCTGGATT TTCATTTTTT AATTACTCGT
4981 TACAATATGC TATTTATTTA TATAAAGAGT TTGTGCCCAT TTAACCAGTA AACAAATTTG
5041 TTCAACCGTA ACTTAGCTTC ATCGACTTTT GGCCTCGCCT GGTCAGAATC TAGGGCCGTT
5101 ATCCTATTTA TTTATGATAA ATAAAATTTA ATTATCTTTA ATAAGCTGAA TATGTGGATT
5161 TGTGCTCAAT CTTGGATTCA AGTATGTATT CCTTTTGGTA CCCTGCTTTA TTTTAAGGCA
5221 GATGAAGAGG ATGCCAACAT GACACAATAT CGATTACGAC TGTAACATTA AAGTCAGTTA
5281 TAAATTTTAT GATTAAAATG AAATTTTAGT AGAAAATCGT ATTCTATTCC GCCATTTACA
5341 ATAGCATCCT CTTTAATATC ATTAATCTCA GATAAAACAA ATAATTACAA TGTGAATAGA
5401 ATAATGACTT ACAAAATAAG CACTAAATCT TCAGATGAAC TCTTAACTGA CAACACTATT
5461 TTATAAAATA ATTGAGGTTA TTATGTATAG CACGGCTGTA TTACTCAATA AAATCAGTCC
5521 CACTCGCGAC GGTCAGACGA TGACTCTTGC GGATCTGCAA TATTTATCCT TCAGTGAACT
5581 GAGAAAAATC TTTGATGACC AGCTCAGTTG GGGAGAGGCT CGCCATCTCT ATCATGAAAC
5641 TATAGAGCAG AAAAAAAATA ATCGCTTGCT GGAAGCGCGT ATTTTTACCC GTGCCAACCC
5701 ACAATTATCC GGTGCTATCC GACTCGGTAT TGAACGAGAC AGCGTTTCAC GCAGTTATGA
5761 TGAAATGTTT GGTGCCCGTT CTTCTTCCTT TGTGAAACCG GGTTCAGTGG CTTCCATGTT
5821 TTCACCGGCT GGCTATCTCA CCGAATTGTA TCGTGAAGCG AAGGACTTAC ATTTTTCAAG
5881 CTCTGCTTAT CATCTTGATA ATCGCCGTCC GGATCTGGCT GATCTGACTC TGAGCCAGAG
5941 TAATATGGAT ACAGAAATTT CCACCCTGAC ACTGTCTAAC GAACTGTTGC TGGAGCTATT
6001 ACCCGCAAGA CCGGAGGTGA TTCGGACGCA TTGATGGAGA GCCTGTCAAC TTACCGTCAG
6061 GCCATTGATA CCCCTTACCA TCAGCCTTAC GAGACTATCC GTCAGGTCAT TATGACCCAT
6121 GACAGTACAC TGTCAGCGCT GTCCCGTAAT CCTGAGGTGA TGGGGCAGGC GGAAGGGGCT
6181 TCATTACTGG CGATTCTGGC CAATATTTCT CCAGAACTGT ATAACATTTT GACCGAAGAG
6241 ATTACGGAAA AGAACGCTGA TGCTTTATTT GCGCAAAACT TCAGTGAAAA TATCACGCCC
6301 GAAAATTTCG CGTCACAATC ATGGATAGCC AAGTATTATG GTCTTGAACT TTCTGAGGTG
6361 CAAAAATACC TCGGGATGTT GCAGAATGGC TATTCTGACA GCACCTCTGC TTATGTGGAT
6421 AATATCTCAA CGGGTTTAGT GGTCAATAAT GAAAGTAAAC TCGAAGCTTA CAAAATAACA
6481 CGTGTAAAAA CAGATGATTA TGATAAACAT GTAAATTACT TTGATCTGAT GTATGAAGGA
6541 AATAATCAAT TCTTTATATG TGCTAATTTT AAGATATCGA GAGAATTTGG GGCGACTCTT
6601 AGGAAAAACT CAGGGACAAG TGGCATTGTC GGCAGCCTTT CCGGTCCCCT GGTAGCCAAT
6661 ACTAATTTCA AAAGCAATTA CTTAAGTAAC ATATCTGATA ATGAATACAG AAATGGCGTA
6721 AAAATATATG CCTATCGCTA TACGTCTTCC ACCAGCGCCA CAAATCAGGG CGGCGGAATA
6781 TTCACTTTTG AGTCTTATCC CCTGACTATA TTTGCGCTCA AACTGAATAA AGCCATTCGC
6841 TTGTGCCTGA CTAGCGGGCT TTCACCGAAT GAACTGCAAA CTATCGTACG CAGTGACAAT
6901 GCACAAGGCA TCATCAACGA CTCCGTTCTG ACCAAAGTTT TCTATACTCT GTTCTACAGT
6961 CACCGTTATG CACTGAGCTT TGATGATGCA CAGGTACTGA ACGGATCGGT CATTAATCAA
7021 TATGCCCGAC GATGACAGTG TCAGTCATTT TAACCGTCTC TTTAATACCC CGCCGCTGAA
7081 AGGGAAAATC TTTGAAGCCG ACGGCAACAC GGTCAGCATT GATCCGGATG AAGAACAATC
7141 TACCTTTGCC CGTTCAGCCC TGATGCGTGG TCTGGGGATC AACAGTGGTG AACTGTATCA
7201 GTTAGGCAAA CTGGCGGGTG TATTGGACAC ACAAAATATC CTCACACTTT CTGTCCCTGT
7261 TATATCTTCA CTGTATCGCC TCACGTTACT GGCCCGTGCC CATCAGCTGA CGGTTAATGA
7321 ACTGTGTATG CTTTATGGTT TTTCGCCGTT CAATGGCAAA ACAACGGCTT CTTTGTCTTC

Fig.2.


```
7381  CGGGGAGTTG TCACGGCTGG TTATCTGGTT GTATCAGGTG ACGCAGTGGC TGACTGAGGG
7441  CGGAAATCAC CACTGAAGCG ATCTGGTTAT TATGTACGCC AGAGTTCAGC GGGAATATTT
7501  CACCGGAAAT CAGTAATCTG CTTAATACTC TCCGACCCCG TATTAGTGAA GACATGGCAC
7561  AAAGTAGTGA CCGGGAGCTT CAGGCTGAAA TTCTCGCGCC GTTTATTGCT GCAACGCTGC
7621  ATCTGGCGTC ACCAGATATG GCGCGGTATA TCCTGTTGTG GACTGATAAC CTGCGGCCGG
7681  GCGGCCTGAA TATCGCCGGA TTTATGATGC TGGTGCTGAA AGAGACGCTG AGTGATGAGG
7741  AAACGACCCA ACTGGTTCAA TTCTGCCATG TAATGGCACA GTTATCGCTT TCCGTGCAGA
7801  CACTGCGTCT CAGTGAAGCA GAGCTTTCTG TGCTGGTCAT TTCCGATTTT GTGGTACTGG
7861  GTGCGAGAAG CCAACCGCCG GACAACACAA TATTGATACT CTGTTCTCAC TCTACCGATT
7921  CCACCAGTGG ATTAATGGGC TGGGAAATCC CGGCTCTGAC ACGCTGGATA TGCTGCGCCA
7981  AGCAGACACT CACGGGCGAC AGACTGGGCC TCCGTGATGG GGCTGGACAT CAGTATGGTA
8041  ACGCAGGCCA TGGGTTCCCG CCGGCGTGAA CCAACTTCAG TGTTGGCAGG ATATCAACCC
8101  CGTGTTGCAG TGGATACATG TGGCATCAGC ACTGCTCACT GATGCCGTCG GTTATCCGTA
8161  CGCTGGTGAA TATCCGTTAC GTGACTGCAT TAAACAAAGC CGAGTCGAAT CTGCCTGCCT
8221  GGGATAAGTG GCAGACGCTG GCAGAAAATA TGGCAGCCGG ACTGAGTACA CAACAGGCTC
8281  AGACGCTGGC GGATTATACC GCAGAGCGCC TGAGTAACGT GTTGTGCAAT TGGTTTCTGG
8341  CGAATATCCA GCCAGAAGGG GTGTCCCTGC ACAGCCGGGA TGACCTGTAC AGCTATTTCC
8401  TGATTGATAA TCAGGTCTCT TCTGCCATAA AAACCACCCG ACTGGCAGAG GCCATTGCCG
8461  GTATTCAGCT CTACATCAAC CGGGCGCTGA ACCGGATAGA GCCTAATGCC CGTGCCGATG
8521  TGTCAACCCG CCAGTTTTTT ACCGACTGGA CGGTGAATAA CCGTTACAGC ACCTGGGGCG
8581  GGGTGTCGCG GCTGGTTTAT TATCCGGAAA ATTACATTGA CCCGACCCAG CGTATCGGGC
8641  AGACCCGGAT GATGGATGAA CTGCTGGAAG ATATCAGCCA GAGTCAGCTC AGCCGGGACA
8701  CGGTGGAAGA GGCCTTTAAA ACTTACCTGA CCGCTTTGAA ACCGTGGCAG ACCTGAAAGT
8761  TGTCAGCGCT ATCACCGACA ACGTCAACAG CAACACCGGA CTGACCTGGT TTGTCGGCCA
8821  AACGCGGGAG AACCTGCCGG AATATTACTG GCGTAACGTG CATATATCAC GGATGCAGGC
8881  GGGTGAACTG GCCGCCGATG CCTGGAAAGA TTGGACGAAG ATTGATACAG CGGTCAACCC
8941  ATACAAGGAT GCAATACGTC CGGTCATATT CAGGGAACGT TTGCACCTTA TCGTGGGTAG
9001  AAAAAGAGGA AGTGGCGAAA AATGGTACTG ATCCGGTGGA AACCTATGAC CGTTTTACTC
9061  TGAAACTGGC GTTTCTGCGT CATGATGGCA GTTGGAGTGC CCCCTGGTCT TACGATATCA
9121  CAACGCAGGT GGAGGCGGTC ACTGACAAAA AACCTGACAC TGAACGGCTG GCGCTGGCCG
9181  CATCAGGCTT TCAGGGCGAG GATACTCTGC TGGTGTTTGT GTACAAAACC GGGGTGAGTT
9241  ACCCGGATTT TGGCGACAAC AATAAAAATG TGGCAGGCAT GACCATTTAC GGCGATGGCT
9301  CCTTCAAAAA GATGGAGAAC ACAGCACTCA GCGTTACAGC CAACTGAAAA ATACCTTTGA
9361  TATCATTCAT ACTCAAGGCA ACGACTTGGT AAGAAAGGCC AGCTATCGTT TCGCGCAGGA
9421  TTTTGAAGTG CCTGCCTCGT TGAATATGGG TTCTGCCATC GGTGATGATA GTCTGACGGT
9481  GATGGAAAAC GGGAATATTC CGCAGATAAC CAGTAAATAC TCCAGCGATA ACCTTGCTAT
9541  TACGCTACAT AACGCCGCTT TCACTGTCAG ATATGATGGC AGTGGCAATG TCATCAGAAA
9601  CAAACAAATC AGCGCCATGA AACTGACGGG GTTGGATGAA AGTCCCAGTA CGGCAATGCA
9661  TTTATCATCG CAAATACCGT TAAACATTAT GGCGGTTACT CTGATCTGGG GGGCCCGATC
9721  ACCGTTTTTA TTAAAACGGA AAAACTATAT TGCATCAGTT CAAGGCCACT TGATGAACGC
9781  AGATTACACT AGGCGTTTGA TTCTAACACC AGTTGAAAAT AATTATTATG CCAGATTGTT
9841  CGAGTTTCCA TTTTCTCCAA ACACAATTTT AAACACCGTT TTCACGGTTG GTAGCAATAA
9901  AACCAGTGAT TTTAAAAAGT GCAGTTATGC TGTTGATGGT AATAATTCTC AGGGCTTCCA
9961  GATATTTAGT TCCTATCAAT CATCCGGCTG GCTGGATATT GACACAGGTA TTAACAATAC
10021 TGATGTCAAA ATTACGGTGG TAGCTGGCAG TAAAACCCAC ACCTTTACGG CCAGTGACCA
10081 TATTGCTTCC TTGCCGGCAA ACAGTTTTGA TGCTATGCCG TACACCTTTA AGCCACTGGA
10141 AATCGATGCT TCATCGTTGG CCTTTACCAA TAATATTGCT CCTCTGGATA TCGTTTTTGA
10201 GACCAAAGCC AAAGACGGGC GAGTGCTGGG TAAGATCAAG CAAACATTAT CGGTGAAACG
10261 GGTAAATTAT AATCCGGAAG ATATTCTGTT TCTGCGTGAA ACTCATTCGG GTGCCCAATA
10321 TATGCAGCTC GGGGTGTATC GTATTCGTCT TAATACCCTG CTGGCTTCTC AACTGGTATC
10381 CAGAGCAAAC ACGGGCATTG ATACTATCCT GACAATGGAA ACCCAGCGGT TACCGGAACC
10441 TCCGTTGGGA GAAGGCTTCT TTGCCAACTT TGTTCTGCCT AAATATGACC CTGCTGAACA
10501 TGGCGATGAG CGGTGGTTTA AAATCCATAT CGGGAATGTT GGCGGTAACA CGGGAAGGCA
10561 GCCTTATTAC AGCGGAATGT TATCCGATAC GTCGGAAACC AGTATGACAC TGTTTGTCCC
10621 TTATGCCGAA GGGTATTACA TGCATGAAGG TGTCAGATTG GGGGTTGGAT ACCAGAAAAT
10681 TACCTATGAC AACACTTGGG AATCTGCTTT CTTTTATTTT GATGAGACAA AACAGCAATT
10741 TGTATTAATT AACGATGCTG ATCATGATTC AGGAATGACG CAACAGGGGA TCGTGAAAAA
10801 TATCAAGAAA TACAAAGGAT TTTTGAATGT TTCTATCGCA ACGGGCTATT CCGCCCCGAT
10861 GGATTTCAAT AGTGCCAGCG CCCTCTATTA CTGGGAATGT TCTATTACAC CCCGATGATG
10921 TGCTTCCAGC GTTTGCTACA GGAAAAACAA TTCGACGAAG CCACACAATG GATAAACTAC
10981 GTCTATAATC CCGCCGGCTA TATCGTTAAC GGAGAAATCG CCCCCTGGAT CTGGAACTGC
11041 CGGCCGCTGG AAGAGACACT CCTGGAATGC CAATCCGTTG GATGCCATTG ATCCGGATGC
11101 CGTCGCACAA TATGACCCGA CACACTATAA AGTTGCCACC TTTATGCGCC TGTTGGATCA
11161 ACTTATTCTG CGCGGCGATA TGGCCTATCG CGAACTGACC CGCGATGCGT TGAATGAAGC
```

Fig.2.

11221 CAAGATGTGG TATGTGCGTG CTTTGGAATT GCTGGGTGAT GAGCCGGAGG ATTACGGCAG
11281 CCAACAGTGG GCCGCACCGT CTCTTTCCGT GGCGGGCAAC CACACTGTGC AAGCGGGCTA
11341 TCAACAAGAC CTTACGGCGC TAGACAACGG AGAAGGTTGC ACTCAACCCC GCAACGCTAA
11401 CTCGTTGGTG GTTTGGTCCT GCCGGAATAT AACCCGGAAT CAACCGATTA CTGGCAAACC
11461 TGCGTTTGCG CCTGGTTAAC CTGCGCCATA ATCCTTCCAT GACGGGCAAC CGTTATCGCT
11521 GGCGAATTAC GCGAGCCTAC GATCCGAAAG CGCTGCTCAC CAGTATGGTA CAGCCTTCTC
11581 AGGGCGGTAG TGCAGTGCTG CCCGGCACAT TGTCGTTATA CCGCTTCCCG GTGATGCTGG
11641 AGCGGGCCCG CAATCTGGTA GCGCAATTAA CCCAGTTCGG CACCTCTCTG CTCAGTATGG
11701 CAGAGCATGA TGATGCCGAT GAACTCACCA CGTTGCTACT ACAGCAGGGT ATGGAACTGG
11761 CGACACAGAG CATCCGTATT CAGCAACGAA CTGTCGATGA AGTGGATGCT GATATTGCTG
11821 TATTGGCAGA GAGCCGCCGC AGTGCACAAA ATCGTCTGGA AAAATACCAG CAGCTGTATG
11881 ACGAGGATAT CAACCACGGA GAACAGCGTG CGATGTCACT GTTTGATGCG GCGGCAGGTC
11941 AGTCTCTGGC CGGGCAGGCG CTCTCAGTAG CAGAAGGGGT GGCTGACTTA GTTCCAAACG
12001 TGTTCGGTTT CGCTTGTGGC GGCAGTCGTT GGGGGGCAGC ACTGCGTGCT TCCGCCTCCG
12061 TGATGTCGCT TTCTGCCACA GCTTCCCAAT ATTCCGCAGA CAAAATCAGC CGTTCGGAAG
12121 CCTACCGCCG CCGCCGTCAG GAGTGGGAAA TTCAGCGTGA TAATGCTGAC GGTGAAGTCA
12181 AACAAATGGA TGCCCAGCTG GAAAGCCTGA AAATACGCGG CGAAGCAGCA CAGATGCAGG
12241 TGGAATATCA GGAGACCCAG CAGGCCCATA CTCAGGCTCA GTTAGAGCTG TTACAGCGTA
12301 AATTCACAAA CAAAGCGCTT TACAGTTGGA TGCGCGGCAA GCTGAGTGCT ATCTATTACC
12361 AGTTCTTTGA CCTGACCCAG TCCTTCTGCC TGATGGCACA GGAAGCGCTG CGCCGCGAGC
12421 TGACCGACAA CGGTGTTACC TTTATCCGGG GTGGGGCCTG GAACGGTACG ACTGCGGGTT
12481 TGATGGCGGG TGAAACGTTG CTGCTGAATC TGGCAGAAAT GGAAAAAGTC TGGCTGGAGC
12541 GTGATGAGCG GGCACTGGAA GTGACCCGTA CCGTCTCGTT GGCACAGTTC TATCAGGCCT
12601 TATCATCAGA CAACTTTAAT CTGACCGAAA AACTCACGCA ATTCCTGCGT GAAGGGAAAG
12661 GCAACGTAGG AGCTTCCGGC AATGAATTAA AACTCAGTAA CCGCCAGATA GAAGCCTCAG
12721 TGCGATTGTC TGATTTGAAA ATTTTCAGCG ATACCCCGGA AAGCTTTGGC AATACCCGTC
12781 AGTTGAAACA AGTGAGTGTC ACCTTGCCGG CGCTGGTTGG TCCGTATGAA GATATCCGGG
12841 CGGTGCTGAA TTACGGCGGC AGCATCGTCA TGCCACGCGG TTGCAGTGCT ATTGCTCTCT
12901 CCCACGGCGT GAATGACAGT GGTCAATTTA TGCTGGATTT CAACGATTCC CGTTATCTGC
12961 CGTTTGAAGG TATTTCCGTG AATGACAGCG GTAGCCTGAC GTTGAGTTTC CCGGATGCGA
13021 CTGATCGACA GAAAGCGCTG CTGGAGAGCC TGAGCGATAT CATTCTGCAT ATCCGCTATA
13081 CCATTCGTTC TTAATTAAAA CATTGTGATA GGCAGGCTCC TGAGGGAGCC TGTTTAAGGA
13141 GTTTTTATGC AGGGTTCAAC ACCTTTGAAA CTTGAAATAC CGTCATTGCC CTCTGGGGGC
13201 GGATCACTAA AAGGAATGGG AGAAGCACTC AATGCCGTCG GAGCGGAAGG GGAGCGTCAT
13261 TTTCACTGCC CTTGCCGATC TCTGTCCGGC GTGGTCTGGT GCCGGTGCTA TCACTGAATT
13321 ACAGCAGTAC TGCTGGCAAT GGGTCATTCG GGATGGGGTG GCAATGTGGG GTTGGTTTTA
13381 TCAGCCTGCG TACCGCCAAG GGCGTTCCGC ACTATACGGG ACAAGATGAG TATCTCGGGC
13441 CGGATGGGGA AGTGTTGAGT ATTGTGCCGG ACAGCCAAGG GCAACCAGAG CAACGCACCG
13501 CAACCTCACT GTTGGGGACG GTTCTGACAC AGCCGCCTAC TGTTACCCGC TATCAGTCCC
13561 GCGTGGCAGA AAAAATCGTT CGTTTAGAAC ACTGGCAGCC ACAGCAGAGA CGTGAGGAAG
13621 AGACGTCTTT TTGGGTACTT TTTACTGCGG ATGGTTTAGT GCACCTATTC GGTAAGCATC
13681 ATCATGCACG TATTGCTGAC CCGCAGGATG AAACCAGAAT TGCCCGCTGG CTGATGGAGG
13741 AAACCGTCAC GCATACCGGG GAACATATTT ACTATCACTA TCGGGCAGAA GACGATCTTG
13801 ACTGTGATGA GCATGAACTT GCTCAGCATT CAGGTGTTAC GGCCCACCGT TATCCTGGCA
13861 AGTCCACTAT GGCAATACTC AGCCGGAAAC CGCTTTTTTC GCGGTAAAAT CAGGTATCCC
13921 TGTTGATAAT GACTGGTTGT TTCATCTGGT ATTTGATTAC GGTGAGCGCT TATCTTCGCT
13981 GAACTCCGTA CCCGAATTCA ATGTGTCAGA AAACAATGTG TCTGAAAACA ATGTGTCTGA
14041 AAAATGGCGT TGTCGTCCGG ACAGTTTCTC CCGCTATGAA TATGGGTTTG AAATTCGAAC
14101 CCGTCGCTTG TGTCGCCAAG TTCTGATGTT TCATCAGCTG AAAGCGCTGG CAGGGGAAAA
14161 GGTTGCAGAA GAAACACCGG CGCTGGTTTC CCGTCTTATT CTGGATTATG ACCTGAACAA
14221 CAAGGTTTCC TTGCTGCAAA CGGCCCGCAG ACTGGCCCAT GAAACGGACG GTACGCCAGT
14281 GATGATGTCC CCGCTGGAAA TGGATTATCA ACGTGTTAAT CATGGCGTGA ATCTGAACTG
14341 GCAGTCCATG CCGCAGTTAG AAAAAATGAA CACGTTGCAG CCATACCAAT TGGTTGATTT
14401 ATATGGAGAA GGAATTTCCG GCGTTACTTT ATCAGGATAC TCAGAAAGCC TGGTGGTACC
14461 GTGCTCCGGT ACGGGATATC ACTGCCGAAG GAACGAATGC GGTTACCTAT GAGGAGGCGA
14521 AACCACTGCC ACATATTCCG GCACAACAGG AAAGCGCGAT GTTGTTGGAC ATCAATGGTG
14581 ACGGGCGTCT GGATTGGGTG ATTACGGCAT CAGGGTTACG GGGCTACCAC ACCATGTCAC
14641 CGGAAGGTGA ATGGACACCC TTTATTCCAT TATCCGCTGT GCCAATGGAA TATTTCCATC
14701 CGCAGGCAAA ACTGGCTGAT ATTGATGGGG CTGGGCTGCC TGACTTAGCG CTTATCGGGC
14761 CAAATAGTGT ACGTGTCTGG TCAAATAATC CGGCAGGATG GGATCGCGCT CAGGATGTTA
14821 TTCATTTGTC AAATAAGCCA CTGCCGGTTC CCGGCAAAAA TAAGCGTCAT CTTGTCGCAT
14881 TCAGTGATAT GACAGGCTCC GGGCAATCAC ATCTGGTGGA AGTTACGGCA AATAGCGTGC
14941 GCTACTGGCC GAACCTGGGG CATGGAAAAT TTGGTGAGCC TCTGATGATA ACAGGCTTCC
15001 AAATTACGGG GAAACGTTTA ACCCCCACAG ACTGTATATG GTAGACCTAA ATGGCTCAGG

Fig.2.

15061	CACCACCCGA	TTTTATTTAT	GCCCGCAATA	CTTACCTTGA	ACTCTATGCC	AATGAAAGCG
15121	GCAATCATTC	TGCTGAACCT	CAGCGTATTG	ATCTGCCGGA	TGGGGTACGT	TTTGATGATA
15181	CTTGTCGGTT	ACAAATAGCG	GATACACAAG	GATTAGGGAC	TGCCAGCATT	ATTTTGACGA
15241	TCCCCCATAT	GAAGGTGCAG	CACTGGCGAT	TGGATATGAC	CATATTCAAG	CCTTGGCTGC
15301	TGAATGCCGT	CAATAACAAT	ATGGGAACAG	AAACCACGCT	GTATTATCGC	AGCTCTGCCC
15361	AGTTCTGGCT	GGATGAGAAA	TTACAGGCTT	CTGAATCCGG	GATGACGGTG	GTCAGCTACT
15421	TACCGTTCCC	GGTGCATGTG	TTGTGGCGCA	CGGAAGTGCT	GGATGAAATT	TCCGGTAACC
15481	GATTGACCAG	CCATTATCAT	TACTCACATG	GTGCCTGGGA	TGGTCTGGAA	CGGGAGTTTC
15541	GTGGTTTTGG	GCGGGTGACG	CAAACTGATA	TTGATTCACG	GGCGAGTGCG	ACACAGGGGA
15601	CACATGCTGA	ACCACCGGCA	CCTTCGCGCA	CGGTTAATTG	GTACGGCACT	GGCGTACGGG
15661	AAGTCGATAT	TCTTCTGCCC	ACGGAATATT	GGCAGGGGGA	TCAACAGGCA	TTTCCCCATT
15721	TTACCCCACG	CTTTACCCGT	TATGACGAAA	AATCCGGTGG	TGATATGACG	GTCACGCCGA
15781	GCGAACAGGA	AGAATACTGG	TTACATCGAG	CCTTAAAAGG	ACAACGTTTA	CGCAGTGAGC
15841	TGTATGGGGA	TGATGATTCT	ATACTGGCCG	GTACGCCTTA	TTCAGTGGAT	GAATCCCGCA
15901	CCCAAGTACG	TTTGTTACCG	GTGATGGTAT	CGGACGTGCC	TGCGGTACTG	GTTTCGGTGG
15961	CCGAATCCCG	CCAATACCGA	TATGAAGGGG	TTGTTACCGA	TTCCACAGTG	CAGCCAAAAG
16021	ATTGTCCTTA	AATATGATGC	GTTAGGATTT	CCGCAGGACA	ATCTTGAGAT	TGCCTATTCG
16081	AGACGTCCAC	AGCCTGAGTT	CTCGCCTTAT	CCGGATACCC	TGCCCGAAAC	ACTTTTCACC
16141	AGCAGTTTCG	ACGAACAGCA	GATGTTCCTT	CGTCTGACAC	GCCAGCGTTT	TTCTTATCAC
16201	CATCTGAATC	ATGATGATAA	TACGTGGATC	ACAGGGCTTA	TGGATACCTC	ACGCAGTGAC
16261	GCACGTATTT	ATCAAGCCGA	TAAAGTGCCG	GACGGTGGAT	TTTCCCTTGA	ATGGTTTTCT
16321	GCCACAGGTG	CAGGAGCATT	GTTGTTGCCT	GATGCCGCAG	CCGATTATCT	GGGACATCAG
16381	CGTGTAGCAT	ATACCGGTCC	AGAAGAGCAA	CCCGCTATTC	CTCCGCTGGT	GGCATACATT
16441	GAAACCGCAG	AGTTTGATGA	ACGATCGTTG	GCGGCTTTTG	AGGAGGTGAT	GGATGAGCAG
16501	GAGCTGACAA	AACAGCTGAA	TGATGCGGGC	TGGAATACGG	CAAAAGTGCC	GTTCAGTGAA
16561	AAGACAGATT	TCCATGTCTG	GGTGGGACAA	AAGGAATTTA	CAGAATATGC	CGGTGCAGAC
16621	GGATTCTATC	GGCCATTGGT	GCAACGGGAA	ACCAAGCTTA	CAGGTCAAAC	GACAGTGACG
16681	TGGGATAGCC	ATTACTGTGT	TATCACCGCA	ACAGAGGATG	CGGCTGGCCT	GCGTATGCAA
16741	GCGCATTACG	ATTATCGATT	TATGGTTGCG	GATAACACCA	CAGATATCAA	TGATAACTAT
16801	CACACCGTGA	CGTTTGATGC	ACTGGGGACG	GTAACCAGCT	TCCGTTTCTG	GGGGACTGAA
16861	AACGGTGAAA	AACAAGGATA	TACCCCTGCG	GAAAATGAAA	CTGTCCCCTT	TATTGTCCCC
16921	ACAACGGTGG	ATGATGCTCT	GGCATTGAAA	CCCGGCATAC	CTGTTGCAGG	GCTGATGGTT
16981	TATGCCCCTC	TGAGCTGGAT	GGTTCAGGCC	AGCTTTTCTA	ATGATGGGGA	GCTTTATGGA
17041	GAGCTGAAAC	CGGCTGGGAT	CATCACTGAA	GATGGTTATC	TCCTGTCGCT	TGCTTTTCGC
17101	CGCTGGCATC	AAAATAACCC	TGCCGCTGCC	ATGCCAAAGC	AAGTCAATTC	ACAGAACCCA
17161	CCCCATGTAC	TGAGTGTGAT	CACCGACCGC	TATGATGCCG	ATCCGGAACA	ACAATTACGT
17221	CAAACGTTTA	CGTTTAGTGA	TGGTTTTGGG	CGAAACCTTA	CAAACAGCCG	TACGCCATGA
17281	AAGTGGTGAA	GCCTGGGTAC	CTGATGAGTA	TGGAGCCAAT	GTGGCTGAAA	ATCAAGGCGC
17341	CCCTGAAACG	GGCGATTACA	AATTTCCCGT	TGGGCAATTT	CCCGGACGTA	CAGAATATTA
17401	ACGGGAAAAG	GCAAAGCCCC	TGCGTTACGT	TTCAAACCGT	ATTCCTGAAA	TAATTTGGGC
17461	AACTATGTCA	AGTTGACCAA	AAAATGCCCG	GCAGGATATG	TATGCCGATA	CCCATTACTA
17521	TGATCCGTTG	GGGCGTGAAT	ATCAGGTTAT	CACGCCAAAG	GCGGGTTGCG	TCGATCCTTA
17581	TTCACTCCCT	GGTTTGTGGT	GAATGAAGTT	GAAAATGACA	CTCCCGGTGA	ATGACAGCAT
17641	AAAGCTCAGT	GATGCCTGTT	CACTGAACAG	ACATCACTCC	ATTTAGGAAT	GAATCATGAA
17701	GAATTTCGTT	CACAGCAATA	CGCCATCCGT	CACCGTACTG	GACAACCGTG	GTCAGACAGT
17761	ACGCGAAATA	GCCTGGTATC	GGCACCCCGA	TACACCTCAG	GTAACCGATG	AACGCATCAC
17821	CGGTTATCAA	TATGATGCTC	AAGGATCTCT	GACTCAGAGT	ATTGATCCGC	GATTTTATGA
17881	ACGCCAGCAG	ACAGCGAGTG	ACAAGAACGC	CATTACACCC	AATCTTATTC	TCTTGTCATC
17941	ACTCAGTAAG	AAGGCATTGC	GTACGCAAAG	TGTGGATGCC	GGAACCCGTG	TCGCCCTGCA
18001	TGATGTTGCC	GGGCGTCCCG	TTTTAGCTGT	CAGCGCCAAT	GGCGTTAGCC	GAACGTTTCA
18061	GTATGAAAGT	GATAACCTTC	CGGGACGATT	GCTAACGATT	ACCGAGCAGG	TAAAAGGAGA
18121	GAACGCCTGT	ATCACGGAGC	GATTGATTTG	GTCAGGAAAT	ACGCCGGCAG	AAAAAGGCAA
18181	TAATTTGGCC	GGCCAGTGCG	TGGTCCATTA	TGATCCCACC	GGAATGAATC	AAACCAACAG
18241	CATATTGTTA	ACCAGCATAC	CCTTGTCCAT	CACACAGCAA	TTAGTGAAAG	ATGACAGCGA
18301	AGCCGATTGG	CACGGTATGG	ATGAATTTGG	CTGGAAAAAC	GCGCTGGCGC	CGGAAAGCTT
18361	CACTTCTGTC	AGCACAACGG	ATGCTACCGG	CACGGTATTA	ACGAGTACAG	ATGCTGCCGG
18421	AAACAAGCAA	CGTATCGCCT	ATGATGTGGC	CGGTCTGCTT	CAAGGCAGTT	GGTTGGCGCT
18481	GAAGGGGAAA	CAAGAACAAG	TTATCGTGAA	ATCCCTGACC	TATTCGGCTG	CCAGCCAGAA
18541	GCTACGGGAG	GAACATGGTA	ACGGGATAGT	GACTACATAT	ACCTATGAAC	CCGAGACGCA
18601	ACGAGTTATT	GGCATAAAAA	CAGAACGTCC	TTCCGGTCAT	GCCGCTGGGG	AGAAAATTTT
18661	ACAAAACCTG	CGTTATGAAT	ATGATCCTGT	CGGAAATGTG	CTGAAATCAA	CTAATGATGC
18721	TGAAATTACC	CGCTTTTGGC	GCAACCAGAA	AATTGTACCG	GAAAATACTT	ACACCTATGA
18781	CAGCCTGTAC	CAGCTGGTTT	CCGTCACTGG	GCGTGAAATG	GCGAATATTG	GCCGACAAAA
18841	AAACCAGTTA	CCCATCCCCG	CTCTGATTGA	TAACAATACT	TATACGAATT	ACTCTCGCAC

Fig.2.

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18901 TTACGACTAT GATCGTGGGG GAATCTGACC AGAATCGCAT AATTCACGAT CACCGGTAAT
18961 AACTATACAA CGAACATGAC CGTTTCAGAT CACAGCAACC GGGCTGTACT GGAAGAGCTG
19021 GCGCAAGATC CCACTCAGGT GGATATGTTG TTCACCCCCG GCGGGCATCA GACCCGGCTT
19081 GTTCCCGGTC AGGATCTTTT CTGGACACCC CGTGACGAAT TGCAACAAGT GATATTGGTC
19141 AATAGGGAAA ATACGACGCC TGATCAGGAA TTCTACCGTT ATGATGCAGA CAGTCAGCGT
19201 GTCATTAAGA CTCATATTCA GAAGACAGGT AACAGTGAGC AAATACAGCG AACATTATAT
19261 TTGCCAGAGC TGGAATGGCG CACGACATAT AGCGGCAATA CATTAAAAGA GTTTTTGCAG
19321 GTCATCACTG TCGGTGAAGC GGGTCAGGCA CAAGTGCGGG TGCTGCATTG GGAAACAGGC
19381 AAACCGGCGG ATATCAGCAA TGATCAGCTG CGCTACAGTT ATGGCAACCT GATTGGCAGT
19441 AGCGGGCTGG AATTGGGACA GTGACGGGCA GATCATTAGT CAGGAAGAAT ATTACCCCTA
19501 TGGGGGAACC GCCGTGTGGG CACCCGAAAT CAGTCAGAAG CTGATTACAC AAGCCGGCGT
19561 TATTCTGGCA AAGAGCGGGA TGCAACAGGG TTGTATTACT ACGGCTATCG TTATTATCAA
19621 TCGTGGACAG GGCGATGGTT GAGTGTAGAT CCTGCCGGTG AGGCCGATGG TCTCAATTTG
19681 TTCCGAATGT GCAGGAATAA CCCCATCGTT TTTTCTGATT CTGATGGTCG TTTCCCCGGT
19741 CAGGGTGTCC TTGCCTGGAT AGGGAAAAAA GCGTATCGAA AGGCAGTCAA CATCACGACA
19801 GAACACCTGC TTGAACAAGG CGCTTCCTTT GATACGTTCT TGAAATTAAA CCGAGGATTG
19861 CGAACGTTTG TTTTGGGTGT GGGGGTACAA GTCTGGGGGT GAAGCGGCCA CGATTGCAGG
19921 AGCGTCGCCT TGGGGGATCG TCGGGGCTGC CATTGGTGGT TTTGTCTCCG GGGCGGTGAT
19981 GGGGTTTTTC GCGAACAACA TCTCAGAAAA AATTGGGGAA GTTTTAAGTT ATCTGACGCG
20041 TAAACGTTCT GCTCCTGTTC AGGTAGGCGC TTTTGTTGTC ACATCGCTTG TGACGTCTGC
20101 ACTATTTAAC AGCTCTTCGA CAGGTACCGC CATTTCCGCA GCAACAGCGG TCACCGTTGG
20161 AGGATTAATG GCTTTAGCCG GAGAACATAA CACGGGCATG GCTATCAGTA TTGCCACACC
20221 CGCCGGACAA AGTACGCTGG ATACGCTCAG GCCCGGTAAT GTCAGCGCGC CAGAGCGGTT
20281 AGGGCACTAT CAGGCGCAAT TATTGGCGGC ATATTACTTG GCCGCCATCA GGGAAGTTCT
20341 GAGCTGGGTG AACGGGCAGC GATTGGTGCT ATGTATGGTG CTCGATGGGG AAGGATCATT
20401 GGTAATCTAT GGGATGGCCC TTATCGGTTT ATCGGCAGGT TACTGCTCAG AAGAGGCATT
20461 AGCTCTGCCA TTTCCCACGC TGTCAGTTCC AGGAGCTGGT TTGGCCGAAT GATAGGAGAA
20521 AGTGTCGGGA GAAATATTTC TGAAGTATTA TTACCTTATA GCCGTACACC CGGTGAATGG
20581 GTTGGTGCAG CCATTGGCGG GACAGCCGCG GCCGCTCATC ATGCCGTTGG AGGGGAAGTT
20641 GCCAATGCCG CTAGCCGGGT TACCTGGAGC GGCTTTAAGC GGGCTTTTAA TAACTTCTTC
20701 TTTAACGCCT CTGCACGTCA TAATGAATCC GAAGCATAAC AATCATGTTC ATTCCCACTT
20761 TGTCATGGAT GACAAGGTGG GTTTTTCGGA TGTGTGGACA GAGACCCGTA CAGGGTCTCT
20821 GTCCAGTTAA TTTTTGGATC AAGAACGAAT GGTGTAACGG ATATGCAAAA TGATATCGCT
20881 CAGGCTGAGC AATAAGCTTT TCTGTTTACC ACTGATACCG GGAAAACTGA GGGTTAATGT
20941 GCCTGTATCG GCCACAGGAA GCCCTTCAAA TGGCAGGTAC TTAGCATCAT TGAAATCCAT
21001 CTGGAATTGA CCACTGTCAT TCATGCCATG TGAGATCACA ATCGCTTTGC AGCCACGTGG
21061 CATCATTGTA CTGCCGCCAT AACTCAGTAT TGCCCGGACA TCCTGATAAG GCCCTAAAAG
21121 GGCAGGTAAC GTCACACTGA TTTGTTTGAT ACGGCGTGTA TTACCTAAAC CGTCAGGATA
21181 ATCGGTAGCA ATATTCAGAT CCGATAATTT GAGGCTGGCT TGCAGTTGTG TCCCTTCGAC
21241 GTTCAAACCG TTAAGCGTTG TGCCTGCACT GCCTTCACCT GCATTGACTA ACTCAGTCAC
21301 TTTATCTTTT AAAATGAAAC TATTTTCTGT CAGACCAGCA TACACTTCAG CCAGAGAAAC
21361 GGTTCTGGTG ACCTCCAGTG CCCGTTCATC TTTTTCCAAA TAGCTTTTTT CCATCTGTGC
21421 TAAATTCAGC ATCAGGGTTT CACCCGCTAA TAAACCCGCA TAAGTCCCAT GCCAAGCACC
21481 TGGTTTAATA AAGTGTGCTG CCGCATTATT CAATTCATAC TGATAAGTTT GCTCTGCCAT
21541 TAAACAGAGT GAGACCGCCA AATCATAAAA CTGATAATAA ATAGCGGACA ACGTTCCACG
21601 GAGCCAGTTG TATAGCGCTG CATTACTGAA TTTACTTTGC AGAAAGGCTA ACTGCGCCTG
21661 AGTTTGTGCC TGCTGAGTTT CCAGATAGTT TTTTTGTAAT ACTGCCGCTT CACGACGTAC
21721 AGCCAGCGTC GCTAATTGAG CATCAATTTG TTTTATCTCA GCTTCCGCAT TATTGCGCTG
21781 AATTTCCCAC TCTTGCCGAC GGCGACGGTA TATTTCTGAT TGGCTGATTT TGTCTGCGGC
21841 AATACGTGTT GCTGACGCAG AAATTTCGAT ACCAATCGCA CTGGCATTGA AAAGCGCCCC
21901 AAAACGGGAA CCTCCCACAG CAAAACCGTA AATATTGGGG ACGAGATCTG CCGCGGCGGC
21961 GGCCATATGC AGGGCTGTGC CGCTGGTGCT CAAGACCGAT GAAGAGAGGT AAAGATCCAT
22021 CGCTTGTTTT TCACCAGCGT TAACATCTTC GTCGTACAGC GTATTGAAAC TGTCAAAACG
22081 AGACTGTGCA CCATGACGGC TTTCTTGAAG CGCCAATTTA TCAGCATCAA TTTCAGCCAT
22141 GACCTTATCC TGCATTTTAA TACTTTGCAG GGCTAACTCA CTGCCTTGAG TTTGCAGTAT
22201 TTCAGCCAAG GCTTCTGCAT CCTGCCGTTC AGTAATGCTG AGCAGGGTAT TGCCAAATTG
22261 TATCAACTGG CTTACCCCCC ACTTGGCATT TTCCAGAATC ACCGGAAAAC GGTACATCGG
22321 CATCACTGCA TGAGGTAAAT CGCCGCCGCC TTGTGAAGCA GTGATGGCAG CACTGAGTAA
22381 CATGGACGGA TCTGCGGGCG TGGCATAGAG AGATAATGAC AGTGGCTGAC CGTCGATTGT
22441 CAGGTTATGG CGTAAGTTAT AGAGGCGTTG CGTCAATGTC TGCCAGTAAC CTTGCAGTTT
22501 TTTATTAATT TGAGGGAGGA ACAATGCGGT TAACGAAATT TGCCGTACGT TTCGTGGGTA
22561 ATGCAGCGCG CTGACGCAGT TGCAGCATTT TATGTTGATA ATGATGCCGC ATTGTTTGGC
22621 TGGCAGCTTC TTCCAGCCGT GGCTCTGACC AATCGTTATC CAATGAAAAA TAAGGCTCAT
22681 CACCCAATAA AGTGAGCGCC TGTACATACC ACATTTTAGC TTCGTTTAAG GTATCACGTT
```

Fig.2.

22741	CAAGCTGGCG	ATAGGCGCTA	TCTCCGCGGG	TAATCAACAA	ATCCAGCATT	TTCATAAAGG
22801	TAGCCACTTT	ATAGTGCATC	GGATCATGCT	GGGCAACGGC	GTCCGGATCG	ACCGAATCCA
22861	GCGGATTGGC	ATTCCAGGAC	GTATCTTCCT	CCAATGGGCG	GACGTTCCAG	TAATAATCCT
22921	GCATTTCACC	CTGAACCGAA	TATCCGGTCG	GGTTCAGATA	TAGCGCAGCC	AGCGTGTCGA
22981	TCCGGTAAAA	TCTGCTCTTG	CAATAAGCGC	TGGAATACCA	TCATGGGCGT	TGTAATAGAA
23041	CAATCCCAAG	AAATAGATTG	CATTGGCGCC	GTTTGAAATC	CATGGGTTCA	GTGTTATTTT
23101	TCATGACACG	ACTTGAATAC	CCCTTTTATA	TTTTTTGATA	TTTTTTACTA	TCCCCTGTTG
23161	TGTCATTCCC	GAATCATGAT	CGGCATCATT	AGTGAATATA	AATTGATTTT	TCGTCTCATC
23221	AAAATAAAAG	AAAGCAGATT	CCCAGGATTT	GTCATAGATA	ATTTTTTTGT	ACCCAACCCC
23281	TAATCTGACA	CCTTCACGTA	TGTAATATCC	TTTAGCATAG	GGAACAAAGA	GCGTTACTGT
23341	GGTTTCAATA	TCAGATAACA	TTCCTTCGTA	ATAAGGTTGT	CTGGCAGAAT	TGCCATCAAT
23401	ATTCCCAATA	TGGATCTTAA	ACCAACGTTC	ATCACCATGC	TCCTCTTTAT	TGTAGGGGGG
23461	CAACTTAAAT	GTCGCATAAA	ACCCTTCACC	TAATTGCGGC	TCTGGTAAAT	TTTGCGTTTC
23521	CATACTTAAA	ACATTATCAA	TACCAATATT	GGCTCTTTCA	GCTAATTTTC	TGGAAAATAA
23581	AGTATTTAAC	CGGGTTCTGT	AAGGGCCAAT	CTGCATATAT	TGTGTGCCTG	ATGGCATTTT
23641	ATGCAGTGAT	ATAACGTTAC	TTGTATCTTT	GGATTTTAGT	TTTATATGAA	TTGGCGATTC
23701	AATAACAATA	TCGTTATAAC	CGCCGTCGGG	TTGCTTAATA	ATAAACTCGC	TCACCAGAGG
23761	AATATCATAG	CCTTCAATAT	CAACTTTTAC	TTGATTAAAA	TCATATACCA	TAGGGTCAGA
23821	TTCGTGTGAA	GGTTTAGATG	CCACATGGTC	TTCAGCATTT	AACTCCACTA	GAATATCAGA
23881	GCCATTTTTT	AATAAAAAAC	TAATGTTTTT	ATCTTGGATC	TGTTCGATCA	TAGATGAAGC
23941	AAGTTTTATT	ATCTGTGGCT	GGTTGAACAT	AAATACACCC	ATGGATCCTC	GCGAAGGAAC
24001	AGTGCCGCAA	TATTTCCCAT	GTTATTAATG	ATTGAAACAT	CATTAGTAAA	TGATTCACAT
24061	ATAGTATGCC	ATACTCCTGT	GTTATCTTTC	CAATCTAATA	CTATGTTAGT	ATCAAGTTTG
24121	AATTCAGCAT	CATCTGATTC	ATAATCATAA	TTTATACCAA	CTCCAATTTC	TGATTTTCTA
24181	GGAATTTTTT	CCTTGGTTCT	TAGATGCATT	AACACTCTAA	AATATTCGGC	ATTTTTAAGA
24241	TCGATGGAAA	TAATAAAATC	CAAAGTTCCA	TAATGAAAAA	CTTCTTCTTC	TTTTCCAAGC
24301	ATTTCATCAT	GTCTATCATA	ATCAAATAAA	ATAACCGTTT	CATCTTCTAC	CATCGATAAC
24361	AGGTATTTAA	CCTCATCATT	ATATATATTG	CCTTTTGAAA	AATTAATTTC	CATTGAAGGA
24421	TTGAACGTTA	AATTAATATG	ACCATTTCCT	GGTGATATAT	ACGAGAGATC	AAAAATATTT
24481	CCGGTAAAAC	TGGCTAATTT	ATTTTTTGTG	GTTATAGATT	CCTTATATTC	GGCCAAATAA
24541	TCTGTAGCAA	ATTGATTGTT	GACTTTGTAT	TCTGTCCTGG	TATCAAGTTC	TGATAATGTG
24601	CTCTTAACAA	TGGCGTCTAA	ATCATTTTCT	GTGAGAATGG	ATAATGTCAT	ATCAGGGTTA
24661	ATGGTCATCC	CTTCTCTTGC	AGGAAGACTA	TTAAAAGAAT	AATTGTCTTT	TTTCTCATGG
24721	AAATAAACAA	TAATGACGTC	TTTTTCATAA	TCAGAAGAAC	AATACATACC	AATGCTGGCT
24781	TTTTATTGA	TCAGGTTTTC	TATTTTATCA	GTCACATTAA	AATTAAACGG	TGAGCTCCAG
24841	CTGCCATCAT	AACGAATATG	TGACAGTTTT	AATATATAAT	CAGTGATATC	TATCTTGCCA
24901	TCTTCACTTT	CATTTTTCAG	CTCTTTTTGT	TCCAGCCACA	GTAAATACAA	ACGAGACTTG
24961	TAAATAACAG	GTCTGATATT	TTCCTGCCAT	ACATTGATGG	GTATTTCAAT	TTTTTTCCAT
25021	TCTCCCCAGG	CATTGGCAGC	AAATTGACCG	TGCTGGCACT	TTTGGTGATC	GACATTGCGC
25081	CAATAATATA	TTCTGGGTTC	TGTCTGGCTA	TAACCAATTA	AATAAGTGAG	CCCCTCATTG
25141	ACATTAATAC	TGTCATGATA	TCCGCTAATC	ACCTGCAAGT	TAGCGACATC	TTCAAATGCG
25201	GTCAGATAAT	TTTTAAAGCT	ATCTTCAACG	GTATCGATAT	TTAACTGACT	TTGGGAAAGT
25261	TGCTGTAACA	GGTTGTTCAT	CATACCTGTC	TGACCAATAC	GAATCGTGGG	GTCGATATAG
25321	TTTTCCGGAT	AATAGGCCAG	TTCAGATACG	CCGGCCCAGG	TGCTATACCG	TCGATTGTAG
25381	GTTTCCCAGT	CGCAGAAGAA	CTGACGGGTT	TTCACTGGCT	TTGATACTTT	TCCTTCAACA
25441	TTATTCAACG	CCCGGTTGAC	ATATAACTGA	ATGCTGGCAA	TGGCTTCTGC	CACACGGGTG
25501	GTTTTCACTT	GGGCAGAAAC	TTGGTTATCA	ATCAGCAGAT	AGCTGTACAA	CTCATCCCGG
25561	CTCTTAATCT	GTTGAGGTGC	ACCATTTTTG	ATGTAGTAAG	CACTGGCCGC	TGTCGTCGTG
25621	GCTTCATCCA	GCCATGCCTG	AAGCTGGTCG	GATTGTTGAC	TGTTCAGTCC	CGCCTGCAAC
25681	AAAGTACTGG	CGGCTTGCCA	ATCATCAAAT	GTTGGCATCG	GGGTTTCCGG	TTCACCGACA
25741	TATTTTAATT	TTATGAGTGC	AGCAACACCA	TCCGGGGTAA	TACCCAATGT	AGCAGCGACA
25801	TCCAGCCATT	GCAGAGTGAC	ATCTATAAGT	TCTCCAGTTG	GTAAAGGTAT	TCACTCCCAA
25861	ACCGGTCTGT	TGCAATGCTT	GTGTCACAAC	CTGAGCATCA	AAATTTTAAC	GCCACCGCCA
25921	AATTGTTCGG	CAGTCAACGC	TCCTAAGTTC	CAAATGCTGT	TAAGATTCTG	TCGCGTAGCT
25981	TCACAACGCA	TGATCACAGC	ATGGAAGCGG	GTCAGCGCTT	GCAAAGTGGG	GAGATCATGT
26041	TGCAGTGCTG	TGGTTTCTGA	TTGGAATTTC	TCCGGTTTTG	TCACCAACAG	GGTCAGTTCG
26101	TTTTCGCTGA	GTCCAATATT	GCGCACAATC	AGAGAAAGTT	GCCCCAGTAC	CTGACAAAAA
26161	GCCACCATGT	TGCTGGTTTC	ATTCTCTGAG	CGATCACGGT	TAGCCGCAAT	AATCATGAAA
26221	TCATCGAATG	TCAGTCCTTG	TGGTTTTATC	TGATTAATCC	ACAGCAAAAT	AGTTTCTGCT
26281	GTTTTGGCTG	AATCCATTTG	AATGCTGGCA	GCAATCAGCG	GGGCAGCTGC	ACGGATCAGT
26341	TCGTCATCAC	CGAGTGAAAG	TGTTGATAAT	CCATTACTTA	GTGTCGTGAT	AAGGTTTTCA
26401	ATATCCGGCG	TAAGGACAGT	GCTGTAATTA	TCCGTGGTCA	TCAGAAACAC	ATCACTGACA
26461	GACCATTTCT	GTGTTGTCAG	CCACTGGGTG	CATTGGAACA	GAAAGCTGAT	TAATTGCGTT
26521	AATGCTGTAT	CAGAAAAAAG	GGCAATTTTC	GTGTTCACAT	AGGGAGAAAC	CGACAACAAC

Fig.2.

26581 ATGGATAATT CATTCACTGT CAGATGATGA ATGTCTGCCA GCAGACGAAC GCGATAAAGC
26641 AGAGACAGGT TCTCGATGGA ACACATAAAT TCTGGATTTG TTCCGCCATT AGCCAGTTTC
26701 CATAATGTAT ACAGTTCAGT ATCATTCACT CTGAAAGCAC GTTTCATTAT TCCCAAATAA
26761 AAATGGTTTT TTGATTCACC GGGGGTTAAA TCCAGTTTGG TATTATCAGC AGAAAACTCT
26821 TGGCCATTTA ATAGCGGTGT ATTGAACAGC ATTGTAAAAT GACTGGGTTG TTGTTTAGTG
26881 GAATATTGGC TGATATCTGA ATGACACAAT ACCAGCGCAT CGCTGACGCT AATATTATAG
26941 TGCTGCATAT AATATTGAAC ATAAAACAGC TTACCCAACA CATTGCTGTC AATGGTTAAG
27001 TCATCATAAA TACTTTCTAT TACTTGCCAG ATATCTTCTG GAGATATGCC TGTGGCTTTA
27061 TACAAACGAA TCGCTTTATT CAGCTTTAAC AGGAATATAT CACCGGGAAC TCCATCATTT
27121 TAAAGTGTGC ATTGGCATTG ATAGCATCCG ACGGATTTGG TTAACTCGCC ATAAGCGGAG
27181 TGTTATACCG TTGGTGATTT GCTCTGTCGT CAATTTAATG GGAATACTGT AATGGGTATT
27241 AGCAATGGGG ACGAAATTTT TATCTTGGTA TATATATTCT TTATCTCCAT TCTGGAGACG
27301 AAAATCCAAG TGGTCAGGTT CTGTTTTTTT TACACTGAAA TTATATTTGT ATTCATTTTC
27361 TTTGATTGGA ATTAGCTCTG CATAGTTTAA ATGTGAATCG TAGAAATCTT TGCGGGTTCG
27421 CTTAATCAAT CTTGCCGTTG CCGTATCATT CCCGTCATTG ACCAATGTTA TCAGTTGCTC
27481 ATTCTTATAC TGTTGATTTG TATTTTTCTT ACCGAAGGAG AGATTGACAA ATAAACTGAG
27541 TTCATCATAA GACAAATCGT AGTAGCGAGC CAAAGAAGCA TAACTCTTAA AAATCAGTAC
27601 ATCATCTGTA CCGAAATTTT TCTTCATCAG TTCTGTTGAA TTTTCCGGTG TAATTTCTTC
27661 TACAAGGATT TGATACAATT CAGGCGATAT ATCAGTCTTA ATAGCCAGTA GCGATGTTGG
27721 GTCCATTAAT TCCGCTACGT CTGTATTACG GCTAAATGCG GTGAGGTTTT TATCTTGCAA
27781 TAAAATTGCC TGACGGGCTG ACTCATACGG CAGATGATAG GGTGTCATGC CGGTTTGCCG
27841 GTAAGTGGAC AACATTTTCA TTACACCGTT ATAGTCAGTT TTCTCTAACG TCTGAATATT
27901 ATGCAGCAGT AATTCATTAG ATAAGGATAA TGTGGAAATT TCTTCATCCA TATTATTCTG
27961 TGTCAGTGCC AGTGAAGCAA TGTCGGGGCG TCGTTTATTC AGGTGATATT GAGAATTGTC
28021 AGGATGAAAA TCTTTCGCTT CCCGATATAA TTCTGTTAAA TAAGCCGCTG GTGAAAATAT
28081 GGAAGCAATT GATCCCGGTT TTACAAAACG GTGGGCGCGG CCATAAAACC AACTGTTGTA
28141 ACTATTGTTT AGGGTTGACG GTGTAATATT AAGGTTAGTG ATATTAGCCA GTTGTGGATT
28201 AGCACGGGAC AAAATGCGCA GTTCTTCAAG TTTATTCTGT TTTGATTCCT GATGAGCCTG
28261 TTGATATAAA AAGTCTGTTT CTCGCCACGT CAGAGTTCCA CTTGTCCTAT GACGAAATTC
28321 GCTGAAAGAC ATAAACGAAA TGTTTGTCAA TAATAAAGTA TCACCAGCCT TTTTCTATTT
28381 ATCTTATCTA ACAGTTCATT AACTTTTATC ATATAAATCC TTAAGTTATT GTCAATTTAA
28441 TGATTAATGG TTTTTAGGTG GAGATTATTA TAATCTGATA GGAATATTAT GGTTAATTAA
28501 ATTGATACTG ATTTATCGCT CTATTCTTTC AATAAAAAAT AAAGAACTTC CCTATAATAC
28561 ATGGATTTAA ATAATGAATA CCGTATGTTA AAAATTAAAT TTTAACAAAC TTTCATGAAA
28621 AAATTCAACT CAACAATTGT TTAAATATTT TTAATTGTGT TTGTGCTGTT TGAAAAATGA
28681 ATGACTAATA TTTATCTATG AAAGATTATT TATTGAGGAT GTCTTGCTTG GTTTCAGGGG
28741 GCTACGTTGG AGTCAGATAA ATGTGTGCAA AAAGAAATCC TTAATAAAGT TGCGTAATTA
28801 CAAAAGTTGG TATATCGTGA CAAGAGTGAT AGTAATGTCA CATAATTTAT TGAATACCCG
28861 AACCTCGCAA ATGCGGGGTT TTTCTTCGCA TAATCAAAGA GAAAGCTATG AAAAAAACAC
28921 TGATTACTCT TATTCTCAGT ACCCTTTCTT TTGGTGCTTT GGCACAGCAG GGTGGCTTCG
28981 TTTCCCCGGA CAGCACAGAC TATACTCAGG GTGGATTTAA AGGTCCAACT CCCAACCTGA
29041 CCAGCGTTGC TCAAGCAAAA TCTTTTCGTG ATGATGCGTG GGTTGTTCTG GAAGGAAACA
29101 TTGTTAAACA GGTTGGTCAC GAACTCTATG AATTCGCGGC CGCATAATAC GACTCACTAT
29161 AGGGATCGCT TATTACGGAC TTATCCGGAA AGCTATCTGG AACCCCTGTT ACGCCTGAAT
29221 AAAACAGAAT TCAGGGATAA CAGTGGTTCT GTTTATGTTG ACATTGATGA TAAGCGCTGG
29281 ATGGGTCTGA CGGCCACTCC AACTGACAAA GTTCGTATCG AAGGTGAAGT GGACAAAGAC
29341 TGGAACAGTG TTGAAATTGA TGTCAAAACT ATCCGCATAG TGAAATAACT CAAGCACTTT
29401 GAATATAGCC CCGCACTCGC GGGGTTTTTT GCTTTCTGGG AGTCGGAAGT TTAACCGTAG
29461 TGACGAGGAT CAAAACTAAG TTAACGGCAG TGGTCACTGA TTTGGTGCAT AAGTTATCAA
29521 AAGTTAAAAA TCAAAACTTA TTTTTTATTT AATAGAGGAA TGTCACCCTG TAGGTGAATA
29581 ACGTTGACGG ATGTAAATAT ACAGTATTAT AGTCCTTTGA TATGTTATTA AATTGAAAAA
29641 CCTTTAAACT ATATTCGGGG GAAATTATTA TGTCAGATGT TCGTAATATT ATTAATGTTG
29701 ATAACAATTT TGGTTGTGAA TATAAAGCGG ATTTATTTAA ATAAGTTTTC ATAATTGTGA
29761 TACACCCATT TTTCTCATCC CCGGTTTTTG CTGTTGTAAG GAAGCGGTTT CCATGAAGAT
29821 TTTGACATGG TTAAGCAACT GCCACATAAA TTGGCAGCAG TGGTTTCGTG TCACGGTTTC
29881 ATGCAAGGAT TGCCATAGAC GTTCAATTTT ATTCAACCAC GGGCAATAGG TCGGTAAAAA
29941 GAGAAGATTA AATTTGGGAT TCTTTGCCAG CCAAACCCTG ACCTTCCGGC TCTTATGAAT
30001 GCAATAGTTA TCTAAAATTA ACGTGATGGT TTTGGCATTA ACATATTGAT TGTTAATTTC
30061 ATCTAACAAT TTGATAAATA AATCTGAGTT CTTTCTCAAG CTACCGACAT AAGTGATTTC
30121 TTTCGTTTTC GCGTTGAGGC AATTGGCAAG GTAGTGTTTT TGGTTCTTTC CGGGGGTAAC
30181 AACACGCTTT TGTTGCCCTT TGAAGCACCA GTCTGCACCG ATTTTCGGGT TCAGGTTGAT
30241 GTCCACCTCA TCCTCATAGA AGACCGGGTG TTTCTCTTGA GGCATTGGAT AACGTCTCGC
30301 TGATTTTTGC CATTTTTTCA TCATACTCAG GGTCAGGCAA TTTTACGGTT GGTGCCGCCC
30361 TTCGCCAAAC GATGCCCGTC CGGCAAAAGT AGCGATAGAG GGTACTTTGA GAGAGCGATG

Fig.2.

30421 TATTCAGTAG CTCATTGATT TTAAGTGTAA TAAGCTCAAG GCTCCATCGT GAACGGAGAT
30481 AGCCAAAATG TTGTGGCGAG TGCTGTAATA AGAAAGAAAT GACTGTGAAG AGCGGAGCTA
30541 AGTTCCAGAT GGCAGGCCTT CCCGCCGGGA GGCTTTTAAG TCCTTCCAAC CCGTATAATG
30601 TTAACCAATT TACCCAACGA TGAACGGAAG AACGTGAACA GTGAAGCGTT CTGGAAACGT
30661 GAGAAACCGT ACTCCCTTCA TGTAACATCA AGAGCGCGGT GAAGCGACGT GCATAGTCCT
30721 TATCCCGGGT TTTCTGGATA GCTTTTTTCA TCGGACGTCG TTCATTTCGG GGTATTGATG
30781 TTATGATTGG CATGACTCAG TCCATTTTGG GATTTGTTTT GATTTGGCGA TTAATCAGAT
30841 CGCGAAAATC GGACTGAGTT CCCTTCAAGT GATCTACTAT TTTGAAATCT TATTTAATCA
30901 GGAGTCAGCA AATGAGTTAT TCCCCATAAT ACCTGACCAT GTGGTTGTTT ATCCGGGAAA
30961 TGATTCATCT ACCGGTGGTA TGTGGATTCC TTGGTGCGAT AGTCAGAAAG ATATTGACTC
31021 TGGCCATTAT ATCAAAGTTA CTTTCAGTAA AAAGGACGCT GCTGATATTG TGAACTACAT
31081 GTTTCAACAT GGCAGTTATG TTTATTTTAC AGACAGTAGT AAACAATTTA GCAATAAGCA
31141 AATTATGTCT GGTGATTCAG CTAAAGGCAA AGGGGATTAT AAGCTTGAAA TTAAAACAAA
31201 CGGGAACCTT CCACTGATGG TATTGAATAA ATATTGATTC ATTATTATTT ATGGATAAGA
31261 AATTAAGTTT ATATTTCATC TGGTTTCTGC AATTAAGTTT TAAAAATTAA TTCTACTTTT
31321 TTTATGGTTT TATATTTAAT GCCAATCATA TTATTTTTCT TATAATAATT GATAGTTTAT
31381 TTATATAGTA AATAAATTCT GTTGGATGTG ATTATTATTG TGAGACGGTA ATAATTAACA
31441 TAACAGAAAA TTCATGGTTA GGAAATTCAA TCAACTTTTG TCCGGTTTCC TGACCATGAA
31501 GAGCTGTATT TACTGTAGAA CTCGCATTGA TACTGGATTG ATTAGCCGGA CGAGTGTTGG
31561 GTCAGCAGAT AATATGTTGT ATATTGGCTG TGGATTTTTC AGCGAGATGA TAGCTTTGGC
31621 AGTAAAGGCG ATTAATAACC GATAAAACAG AGAGACGGAT TGTGGCCAGG AAAGCAAAAA
31681 AGCCTCACCA TGACGCGTTA TTCAAACATT TTTTAACCCA ACCAGAAACC GCCCGGGAAT
31741 TTTTATCCCT TTATCTGCCG GAAGCGATCC GGTCAGTGTG TGATTTACCA CACTAAAACT
31801 GGAACCGGCA GCTTTGTGGA CAGGCAATTA CGTCAGTTGC ACAGTGATGT GCTGTATTCT
31861 GTCGAGACAA CCCACGGGGA CGGTTACATT TATTGCCTGA TTGAACACCA GTCCACGCCT
31921 GATCCGTTAA TGGCCTGGCG GCTGATGTAT TATTCGCTGT CAGCCATGGC TGCGCATCTG
31981 AAAAAAGGAC ATACTGAACT CCCTTTGGTC GTCCCCCTGC TGTTTTATCA TGGTGAGGTG
32041 AGGCCTTACC CTTACTCAAA TCGATGGCTG GATTGTTTTA CACTCTCTGA ACACGCGGCT
32101 CACCTGTATA ATCAGCCCCT GCCGTTGGTG GATATCAGTG CGCTCAGTGA TGAAGAGATC
32161 CTGACACATA AAAGCATTGC CTTGATGGAG CTGGTACAAA AACATATCCG TTGCCGGGAT
32221 ATGCTGGAGT GGGTTCCCCA ATTGGTGGCG TTGTTGAATG CCGGTTATAA TAGCGCCGAA
32281 CAGCGCCATG TTGTGTTAAG CTATATTTTA CTGAATGGAC ATACGCTGGA TCTCGCCCAG
32341 TTTGTCCATC AACTGACTGA ACAATCTCCG GAGCATGAAA CCATGTTGAT GACTATTGCA
32401 GAACAGCTTG AACAAAAAGG GCGTGAGCAA GGCCGGACAG AAGGCAGAAC AGAAGGCAGA
32461 GCTGAAGGAC GGGAAGAAGG CAAGCTGGAA ACGGCGCGCG CATTATTACG GCATGGTGTC
32521 AGTCTGGACA TCATTGTCAC CAGTACCGGC CTGAGCCGGG AGAAAATTGA AGCGTTAAAG
32581 CATTAAATGG ATACGCTTTT TCACAGCAGG ATATGGTGAC CCCTGTGAGG CCACCGGAAA
32641 ATTTTATTTA CTACGATTTA CGACGGGTTA CTTTAGGAAG CTGAATGAGA CGTCCTTTGT
32701 TATATAACGG TCCCATATCA ATCTTCTCTT TTCCGCGTAC AGGTAAGTAA CCCAAACCTT
32761 CGTGAGCAGC ATTTGCCAAC AGGCCATCAT CCTGATCGCC TGACCAAGAG AAGATCCCGC
32821 CCAATTTCAT TTTGGTTGCA TAAATTCCCT TATGCAGCAC AGTGCGGGGC GTATCCAGTG
32881 AAATCCAGTG ACCACCGTCA GCATTAAAGA GTGCGTCAGC GTCGGTTTCC GTGTCTGTCA
32941 CCAGTTCAAA CTGATTTTTC CCGCGTGCAA TTTCATATTC CGCATCGTAT TGGTTATTCA
33001 GCAGACAGAA GAATTCCGGA GCACCTTTTT CCATCGTGCC CAGTGGCTCT CCTGTTCTGT
33061 TATAGCGGCG CGTTGTCAGA TCAGCACCCA GACATGAACG TCCATAGTTA GCAAATCCGA
33121 GGTGAATTTT CTCCGGTTGT ACACCTTGTG ACAGTAAAAA GCGGATCGCC TCATCTGCCG
33181 AGTAATCCAT GTCCCGATCA GGATTGGGCG GAGGAGGGTT ATCGCCGTCA TATTCATATC
33241 TGGGGGGATA CAGGTTAGTA TGGTGACCGA TGTATTCTGC CCAACCGGTA CCAAAGAAGT
33301 CGTAGGTCAT CACAAAGATA TTGTCTAAAT AAGGTGCGAT TTCTTTGAAG CTGGACTTCT
33361 CCATTTTGGC AACGACGGCG CTACAGGCTA TCGTGATTTC TTTACGGGCC CGGGTTCCAA
33421 AGGCGATGTT CAGTGCTTCA CGCAGCTCTT TCACTAACAA AACATAGTTT GGGCCATCAT
33481 GTTCCGGGTC GAATTCATTA CCTTCTTCAC CTGTGGCGCC GGGGTATTCC CAGTCGATAT
33541 CCACCGCAGT AAACATGGGA AAACGCCGGG AAGAAGTCGA CGATGCTACT CACAAATGTA
33601 GCACGTTGCT CAGGATCTTT GGCCATCACA GAGAAATACC CTGACATACT CCAGCCGCCG
33661 ATACTGAATG CGAGTTCCAG CTTATGCCCT GCCTGTTTTG CTCGCGCTTT CAGATTACGC
33721 AATCCCCCCA GTAAACCGGA GGCTGCATCC TGATTGTAAT ATTGCAAGAA ATTCTTCGGG
33781 CTGGCATCAC GGCGCTGATC CGCGTCCAGA CCGACATTGC GTGTGGTGCC TAAATCACCA
33841 TAAGGATCAA CGGGTACAAT ATGGCCTAAT GTAATAGGGG CAATCTGGCC ACTGCTGGCT
33901 TCTGCTTGCC GGTTCCACCC GTCAACAACC TCATTAATCC GTTCGGATAA CTTGCCTTTG
33961 TCACCGTTGA CGGCCATAAA ACTGAAAATC AGGCGGTCGT AGGCGGTAGG CGGGATTTTT
34021 TCCAGATCAA AACCACGGCC GGGGGCATCG TCGCTGGTCA GCGCAGTGTT ATCCTGGGTT
34081 TCTGGCGACA AACGCGCATC ATACTGGCAC CAGTCAGTAA TATAGGCAGA GACTTTAGGC
34141 AGCGGTTCTG TATTTTCCGG ATCAACTTCA TATTCGTTGT ACAGGGACTT GGCAACACGT
34201 GCTGAAGAAT AACTCAAAGG AGTTCCGCTG CCGTCAGGTT TATATCCCAC CTTCTGATAG

Fig.2.

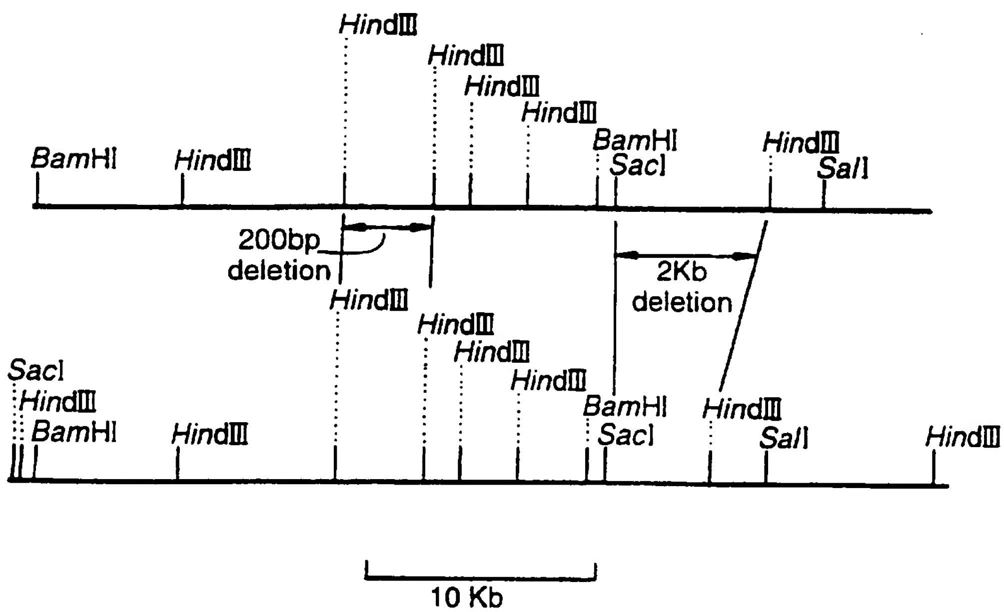
34261	GTTTCTTCTG	TGAGTGCATC	ATATTGCAAT	ACCTCGGTTT	TTTCTCCCGG	CGGTACATCA
34321	GGCGTATTGG	GGTTACCGTG	ATCGGCAATT	TCTTCCGGTG	TCGCCTCACG	GACATATTGC
34381	CAGGCATTCT	CATAAACCGG	TAAATCAGGT	GAAATATTGC	GGTCGGGAAT	ATGCCAGCGT
34441	TCAACCCAGC	CGATGTTTTT	AAAAACCGCG	CTATCATAAA	TGACATACCA	GGTTTGACCA
34501	CCAGATTGAT	TCTGCCAGGC	AACCAGAGAT	GCGCCTACTT	CGCTGCTGGC	GTCAGACATC
34561	GCTTTAATTG	AAGGGTATCG	ATAAACATTT	TGAGACATAA	TTTCACTTCC	GGCCCCGTTA
34621	TATTCCGGGG	CCGGCTCCTG	ATATCAGTTA	GAATTGTCTT	GTTTTAATTG	ATGTTTATTC
34681	AGACGGCTAC	GAACCTGCTG	GCTGAACTCA	TTACTTCCGC	CACTCACATC	ACGCGCGGTA
34741	TAACGCAGAT	GGAGGATAAT	ATCGCTCAGC	GACTCCAGCA	GCTGATCCTG	ATCGGAACCG
34801	AATTCCAACT	TCCACTGTGA	AATGGCGCCT	GTCCCTTCAA	AAGGCAGGAA	AAGTTCATCA
34861	TCAAAATTGA	GCCTGAACAT	GCCGCTGTCT	TCCATGGCCG	TTGAAATCAC	CACACCTTGA
34921	TTAGCCTGTA	CGTTCAGCAA	AACGTTTTCG	GGTTTGGTGT	ATTCCAAGGG	GTTAAGCAAA
34981	TAATCGATAG	TTTTTAAGTC	AGCAGTACTG	TAAAGCGTAT	TGCTGAGTTG	TACCAGTGAA
35041	GCCCGTACAT	CTTCATAAGG	CCCCAGCAAT	GCGGGCAATG	ACAGCGCTAC	GGTTTTTATA
35101	CGCCGATCAG	CGTGGGTCGG	ATAATCGCGC	AAGAACATTT	CGGCGCTCAG	TAAGAAAGTG
35161	AATGAACCCG	TACTCTTGCC	AATTTCCCAC	TGTGATGATG	TCAGTAATGA	TTTTACCGAT
35221	ATGGTTTTTA	TGATCTCCAG	ACGTCTGGTG	TTATGTTGCA	AATACGCCTG	ATCCATCCGT
35281	TGTAAGGCTA	ATTTCAGATG	TTCTCCGACC	AGCAGCCCCT	GATAAAGATC	ATTCCAGAGA
35341	CCACTTTGGA	CGAAATTCAT	ATCATACTGA	CCTGTTTCGT	ACTGCCAGGA	GGCTTCGGCC
35401	AGTAAACAGA	GGGAATTAAC	CGCATCATAG	GCTTGCAGGT	AAAGCCGGAG	ATTTGGCTGA
35461	TCATCCACAT	GTATAACGCA	TCATTGGTAN	ANTTGTTCNN	NNNNNNNNNN	NNNNNNNNNC
35521	CCGAAGCATA	CCGCCAAGAC	CATCCCCCCG	ACGGCCAGAC	CGAAAATATT	GGGAACCATA
35581	TCCGCCACAG	CGGCCGCAGT	GGCGGCTGAC	TGGGCAGCGA	TCACACCTTC	AGCCGCTCTT
35641	GATTGTAATG	CGATAACTTC	CTGCTCGGTG	ATGGAGATGT	TTTCATCATA	GAGCGATTTA
35701	TAGTGTTGCT	GGCGCTCCTG	AGCGGCCCGT	CGGCTGATGG	TCAGTGCATC	CAATGAAGCC
35761	TGTTGCATGT	CAATCGCTTG	CTGTTGCAGA	TTGCGGGTAA	AGCTGTACAG	CCCCAGTTGC
35821	TGCTGCATAC	GGAAGTGTTC	AAAATCGGTA	TTGTCTTTTT	TCTCCAGCAA	ACTCAGTAAC
35881	GTGCTGCCGT	ACTGAATCAG	CGTTTCTGCG	GCCTCTTTTG	CCCGGCTCAT	GATCGGGGTG
35941	AAACGATAAT	TCGGGATTGC	CCGGCGTTTC	ATGCCCGCCA	TACGATTAGC	CACAACACGC
36001	TGGTAACGCT	GCCTGAGCAG	ATCTTGCGGG	CTGATGGGTT	CATCGTATAA	TCCGGCCGGA
36061	AACTCTTTAC	CATCCAAGGT	CAGGTTATGA	CGTAAGTTAT	ATAGACGCTG	ATCCAACATT
36121	TGCCACAGTT	TGAGATATTC	CGTATCAACA	GGTTTGACAA	ATAAATCAGA	CGGTGCGGCA
36181	GAGACGGATG	TATCATATGT	CACAGGCAGA	AGTGGCACGT	TGCTGACAGT	AAGCATTAAC
36241	TCCTGTGCCC	GTGCTTCACT	GTTTTCATAC	AGAGCCACAT	CTTGCAGCGT	ACGGGGTTGC
36301	CAGTTTGCCG	CGAGCAGAAT	ATCAGGGCTG	GTACCCAGTA	ACATATTGAC	GGAGTCATAG
36361	ATCTGCTTGG	CGACAGTACG	TGCACTGGAT	GTCAGCTTAC	GGTATTCCAT	GTCTCCCTGA
36421	TCTAACAGAT	TCTTGACATA	GAAACGGAAT	ATTGCTTTCC	GGTAGTGAAT	GGGTTCACTG
36481	GCTGCAATGG	CATCCGGATC	GGTTGGTTCA	ATTAACATCC	GGTACACGGT	GGGTGGAGGA
36541	TCAATAATTG	GCCGTGAATT	CCAGTAACGC	GGTTTACCTT	GGTTGCTGGC	CTGAACAAGT
36601	TCATCTTCCA	GCGGATTAAA	AATATAGTGC	AGCCATTCGG	TGGCCTCTTT	TAATCGTTGT
36661	TCTATATTCA	GTCGCCACGC	GACCAGAAAT	GGCATATGGA	AAAACAGTTC	CCAGAAATAG
36721	ATCCCATTTG	CGCCATTTAA	ATCAATCGGC	GTAGGGAATG	AACCGGGTAT	AGGCTGTTCG
36781	GTAATAAGCT	GTGTATTCCA	GCTCAGTACC	TGCGGGATAC	CCTGACTGGC	AATGGCGATC
36841	AGTTTTTTTG	CAAACAGTGT	ATTAAGGCGA	ATGTTTTGTG	GCGCGTTATC	AGTTTCATCT
36901	GCGGGGAAGG	AAAGGAATTG	CACCTGATCC	TGTTCATTGA	GTTTAATCAG	TTCGCGAATA
36961	TGCATACCGA	TTCTGAACTC	TTGAGTACAG	CTGGCACTTT	CATTGCCAAC	ACCACCTTTG
37021	GGCTTAAAGA	GAAGTTCGGC	TTTCAGGGTG	ATTCGATTAT	CCGACCCCAG	CTTGATTGAT
37081	GGATAGGTTA	AATCAAGAAC	TTTTTCGCTC	AGTACCAGTG	GTTGTTCATC	CAAGACAGTA
37141	TTATCGTGCA	TCAGCCGGAA	AGAACCGTTG	TAATATTGAT	GATCTTCTAT	CGCACCAAAC
37201	TTAAAGTCAG	ATTGAGCGAC	AATCTCCAGT	GTGTCATCAG	TGCCATGAAC	AAAATTGACA
37261	ATCAGTTTGA	TACTGTCTTT	GCCGAAATCA	GGGTTCATTC	CGGTTTGGAT	TCTCCGGCAA
37321	TAGGAAAGCG	TTCTTCCCGG	GTTGCCGGAT	AGAGCACCAT	AGTACGGTAA	TCGATAGGAT
37381	TGCCTTAAGG	CATCCTTGTG	TTCACGTGAG	TAATACCAGA	CCAGGTTGCC	GACATATTTT
37441	CCTTTTCGTC	CATCAGCATA	TTGGTCATCC	GGCAAATCAG	TAATTTCTAC	CAGCAGTGTA
37501	TCGCAGACAT	AACCGAAGGC	TTCGTCATAA	TCATAATCCT	TACCTTTCTT	ATCTGTCCCC
37561	TGAAGACGGA	CAAACGGAAC	CAGAGCCAGA	AACGGGTTAT	GCGGGTCTTG	CTGTATATCC
37621	ATCACAGCAA	CCATCTGGGC	CATCCGGTAT	TGCAGATGTC	TTCGCGCAGA	ATGGTGGGTG
37681	TACTCCAGCT	GCCATCATAT	TTGGCATAAG	CGATTTTGAT	CCGGTCAGGA	ACGGTGTGGG
37741	AGGAACCCAA	TCACCCGCAC	TAGGCTCAAC	GTTTTGGTTA	TGCAGTGATA	ACGCAGTTGT
37801	ATCTTTAGTT	TCAGACTGTT	CTTCAACTTC	CGTCCAGGCA	ATATACAGGC	GATTATTCAG
37861	GAAAATGGGG	CGTATCAAAT	TGGGGTCTAC	GCTGCCCAAT	GGCAGGTCAA	TAGGTTTCCA
37921	CTCGCTCCAG	GCATTGGGAG	ATAACGCATC	GGTATCAGGA	TGGCGTATCG	AAAGATTCAG
37981	TGAACGCCAG	TAATATTGGT	ATGGCTGTGT	ACGGGTACGT	CCGACAAAGA	AGAACTTATC
38041	GCGTTTGATG	TTAACACCAT	CTTCATAACC	TGCGATAACT	TTCAGGTTAC	TGACATCTTC

Fig.2.

```
38101  AAAATTATTC AGATAACCGA GCACCGCTTG TTGTACAGAA TCTTCGGTAA TTTTTCCCTG
38161  ATTAAGGGCA CTTTCCAGTT GGAAGAAGAA TTCTGTTTTA TTCAGGCGTA ACAGGGGTTC
38221  CAGATAGCTT TCCGGATAAG TCCGTAATAA GCGATCCC
```

N=unspecified base

Fig.3.



INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/02284

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01N63/02 A01N63/00 C12N1/20 C07K14/24 //(A01N63/02, 63:02,63:00),(A01N63/00,63:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01N C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 00647 A (COMMW SCIENT IND RES ORG ;SMIGIELSKI ADAM JOSEPH (AU); AKHURST RAY) 5 January 1995 cited in the application	1,5,11, 13, 18-21, 24-26, 29,30,32
Y	see page 1, line 3 - line 29; claims 10-13	3,4, 6-10,12, 14,27, 28,31

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
17 December 1997	14/01/1998

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European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Muellners, W

INTERNATIONAL SEARCH REPORT

national Application No
PCT/GB 97/02284

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	CHEMICAL ABSTRACTS, vol. 118, no. 1, 4 January 1993 Columbus, Ohio, US; abstract no. 3550, YAMANAKA, SATOSHI ET AL: "Biochemical and physiological characteristics of Xenorhabdus species, symbiotically associated with entomopathogenic nematodes including Steinernema kushidai and their pathogenicity against Spodoptera litura (Lepidoptera: Noctuidae)" XP002048914 see abstract & ARCH. MICROBIOL. (1992), 158(6), 387-93 CODEN: AMICCW;ISSN: 0302-8933, 1992, ---	3,6
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/02284

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	H.MATSUI ET AL. : "Nucleotide sequences of genes encoding 32 KDa and 70 kDa polypeptides in mba region of the virulence plasmid, pKDSC50, of Salmonella choleraesuis " NUCLEIC ACIDS RESEARCH , vol. 18, no. 8, 1990, pages 2181-2, XP002050055 see the whole document ---	21-25
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